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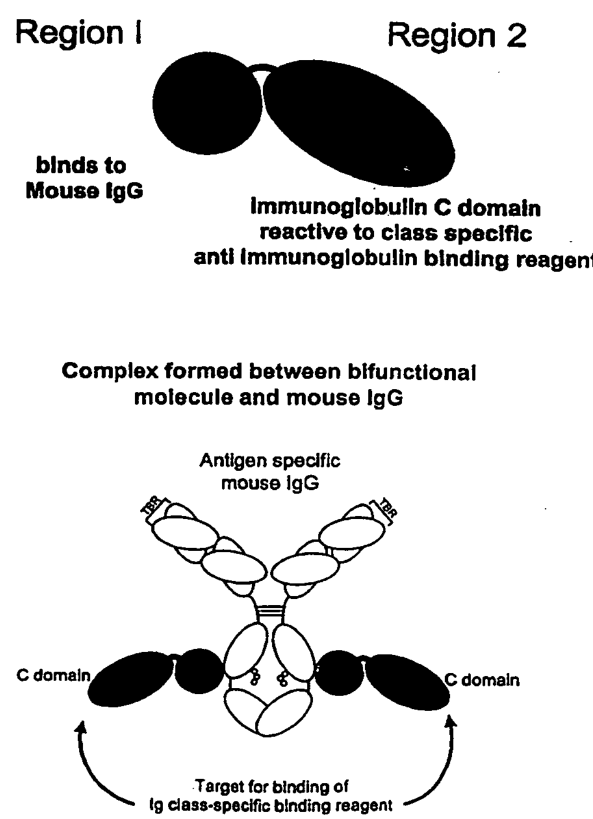
(57) Abstract

A chimeric antibody conjugate comprising an antigen binding region of a non–human antibody and an immunoglobulin constant region which comprises at least one C_H domain or epitope thereof, with the proviso that the constant region is not a naturally occurring F_C fragment. A bifunctional molecule for use in labelling an antibody derived from a first species, the bifunctional molecule comprising a binding region which binds to the antibody of the first species or to one or more groups provided thereon, and a constant region derived from an antibody of a second species, the constant region comprising at least one C_H domain or an epitope thereof. The present invention relates to bifunctional molecules and complexes which are useful as positive control reagents in antibody based diagnostic tests. The present invention also relates to polynucleotides encoding these bifunctional molecules, and to diagnostic assays involving the use of these molecules.



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Bifunctional Molecules

Field of the Invention

The present invention relates to bifunctional molecules and complexes which are useful as a positive control reagents in antibody based diagnostic tests. The present invention also relates to polynucleotides encoding these bifunctional molecules, and to diagnostic assays involving the use of these molecules.

Background of the Invention

Infection of humans by many micro-organisms leads to the initiation of a humoral immune response that can be used in the diagnosis of the disease. In the early acute phase of the infection, specific IgM class antibodies are the first to appear in serum 1-4 weeks after the onset of symptoms and last for up to three months. IgG class antibodies appear later and remain elevated throughout the patient's life. Detection of an IgM response is indicative of a recent or current infection, while the presence of an elevated IgG response is a marker for past exposure to the causative agent. Specific IgM or IgG responses to a particular infectious agent can be measured by antibody based diagnostic tests such as ELISA, immunochromatography, particle agglutination ELISA, biosensor or other similar assays.

These assays require the use of reactive human sera as a positive control. The positive control reagent is usually serum taken from a patient or animal which is known to have a positive reaction to the particular antigen under test. If the test is designed to distinguish between early and late infection (via the differentiation between immunoreactive IgM, for early infection and IgG, for late or previous infection), the positive control serum or reagent should contain immunoreactive antibody of the correct immunoglobulin class.

It is becoming increasingly difficult to source sufficient quantities of immune human sera or plasma, particularly as diagnostic tests for rarer diseases become available. Collection of blood for IgM controls from patients in early stages of infection when clinical symptoms are generally most severe poses significant ethical problems, particularly if the disease primarily affects juveniles. Other drawbacks include the requirement for consistent collections from remote locations, the need to standardise each batch and to

check for contamination with infectious agents such as HIV, hepatitis B and hepatitis C. There are also problems in obtaining control sera for specific endemic diseases in communities where the donation of blood or blood products is socially unacceptable.

There is therefore a need for a source of positive control reagents which does not rely on being obtained from human donors.

Hybridoma technology provides a plentiful supply of monoclonal antibodies, but as these are generally of murine origin, they do react with binding reagents used to quantify human antibodies. Intact, functional mouse/human chimeric antibodies have been described in the literature for some time (Boulianne et al., 1984, Morrison et al., 1984; Winter et al., 1991). In these constructs the antigen binding function residing in a mouse Fab or Fv fragment has been grafted on to a human Ig backbone and expressed in hybridoma cells. In some cases these reshaped molecules have been designed for human therapy, utilising the effector functions of the human Fc for targeting (Reichmann et al., 1988). Others have been designed as positive control reagent substitutes (Hamilton, 1990, 1991), where V_H and V_L regions from a mouse monoclonal antibody of desired specificity have been grafted onto either a human IgG or IgM backbone.

Synthetic positive control reagents are available from a limited number of sources. US 4,929,543 relates to chimeric antibody fragments where Fab or F(ab')2 fragments of non human origin, with specificity for the desired antigen, are chemically coupled to human Fc fragments in order to confer upon the reactive non-human Fab fragments epitopes recognised by class specific anti human immunoglobulin antisera. This reference does not teach or suggest coupling non-human Fab or F(ab')2 fragments to individual C_H domains in order to provide epitopes for recognition by class specific anti human immunoglobulin antisera. Furthermore, production of the chimeric fragments is entirely by synthetic routes based upon digestion of antibodies, purification of fragments and chemical linking to create the chimera.

Labor Diagnostika GmbH of Heiden, Germany have produced synthetic positive control reagents which are formed by chemical attachments of non human Fab fragments and human Fc fragments onto a latex bead. These attachments confer upon the bead the twin properties required of a positive control reagent - specific antigen binding and human immunoglobulin class specific epitopes.

A process for producing positive control reagents which circumvents the requirement to manipulate full length Fc fragments, or to manipulate VH and VL sequences for each new control reagent specificity, is desirable.

Summary of the Invention

The present inventors have now developed bifunctional molecules which may be used as positive control reagents in antibody based diagnostic tests.

In one aspect of the present invention, the bifunctional molecule is a chimeric antibody conjugate comprising a first region which binds a specific antigen and a second region comprising at least one constant domain sequence derived from a class specific immunoglobulin. This conjugate, which may be used directly as a positive control reagent, avoids the inconvenience of manipulating full length or naturally occurring Fc fragments. Furthermore, the conjugate may be readily produced by recombinant DNA technology.

Accordingly, in a first aspect the present invention provides a chimeric antibody conjugate comprising an antigen binding region derived from a non-human antibody and a constant region which comprises at least one C_H domain or epitope thereof, with the proviso that the constant region is not a naturally occurring F_C fragment.

When used herein, "naturally occurring Fc fragment" means a full length naturally occurring Fc fragment which may be derived by proteolytic digestion of an intact antibody molecule. For example, a naturally occurring Fc fragment of IgM will comprise domains C_H2, C_H3 and C_H4, whereas a naturally occurring IgG Fc fragment will comprise C_H2 and C_H3 domains.

By "chimeric" we mean that the constant region is derived from a different species than the antigen binding region.

In a preferred embodiment the non-human antigen binding region comprises or consists of a non-human Fab fragment or part thereof. The non-human antigen binding region may comprise or consist of an scFv fragment.

In a further preferred embodiment the non-human antigen binding region is derived from a mouse.

In a preferred embodiment the constant region is derived from a human antibody. It will be appreciated, however, that the constant region may be a non-human (such as bovine, canine, ovine, equine, feline or

caprine) constant region in cases where the chimeric construct is to be used as a positive antibody control in assays involving sera derived from non-human species.

The constant region may consist of a non-naturally occurring combination of C_H domains or epitopes thereof. The constant region may consist of two C_H domains of the same type, for example, two C_H3 domains. Alternatively, the constant region may consist of two different domains. The two different domains, or epitopes thereof, may be derived from antibodies of different classes. In a preferred embodiment, the constant region consists of a single C_H domain.

In a particularly preferred embodiment of the present invention the chimeric antibody conjugate is suitable as a positive IgM control and the constant region comprises one or more $C_H3\mu$ domains.

In a further preferred embodiment the non-human antigen binding region binds to an epitope derived from an infectious agent selected from but not limited to dengue virus, rubella virus, herpes virus, parvovirus, human glycophorin, *Rickettsia sibirica, Burkholeria pseudomallei, Salmonella typhi* or *paratyphi, Leptospira interrogans, Plasmodium falciparum/vivax,* Japanese encephalitis virus, Yellow fever virus, *Bordetella pertussis/parapertussis, Candida albicans/kruzei,* Varicella zoster virus, HIV, Hepatitis viruses, Human papilloma virus, Epstein-Barr virus, Ross River virus, *Brucella abortis,* Human herpesvirus-6, Parvovirus B19, *Coxiella burnettii,* Herpes simplex viruses 1&2, *Rickettsia rickettsii, Conori australis, Rickettsia tsutsugamushi.*

In a second aspect the present invention provides a recombinant polynucleotide molecule comprising a sequence encoding a non-human V_H region, a sequence encoding a non-human V_L region, a sequence encoding a flexible linker positioned between the V_H region sequence and the V_L region sequence, and a heterologous sequence encoding a C_H domain or epitope thereof.

By “heterologous sequence encoding a C_H domain” we mean sequence encoding a C_H domain which is derived from a different species than the sequences encoding the V_H and V_L regions.

In a preferred embodiment of the second aspect the heterologous sequence encodes a human C_H domain.

By ‘flexible linker’ we mean a region of amino acids of sufficient length and flexibility to allow the V_H and V_L polypeptide regions to dock

correctly with respect to each other to form an scFv fragment. The flexible linker may be a polypeptide of between 12 and 30 amino acids in length. Preferably the linker is a polypeptide of about 15 amino acids in length. The linker may have the sequence GGGGSGGGGSGGGGS.

In a preferred embodiment, the C_H domain sequence is linked to the 3' end of the V_L or V_H sequence. In this preferred construct the chimeric antibody conjugate is synthesized as a single polypeptide chain which folds to produce two separate functional domains.

In a further preferred embodiment of the second aspect of the invention, the polynucleotide molecule includes a control sequence which directs the synthesis of both the V_L and V_H polypeptide regions. The control sequence is preferably an inducible promoter such as the lac promoter.

In a further preferred embodiment the polynucleotide molecule includes a sequence encoding a leader peptide which directs the synthesised polypeptide chains to the host cell periplasm. The leader sequence may be the pel B sequence.

In a third aspect the present invention provides a recombinant polynucleotide molecule comprising a sequence encoding a non-human V_L region, a sequence encoding a non-human C_L region, a sequence encoding a non-human V_H region, a heterologous sequence encoding a C_H domain or epitope thereof and optionally a sequence encoding a non-human C_H1 region.

In a preferred embodiment of the third aspect the heterologous sequence encodes a human C_H domain.

In a further preferred embodiment of the third aspect of the present invention, the V_L and C_L sequences are linked together so that the V_L and C_L regions are expressed as a single polypeptide. In a more preferred embodiment, the V_H and C_H1 sequences are also linked together so that the V_H and C_H1 regions are expressed as a single polypeptide.

In a further preferred embodiment of the third aspect the polynucleotide molecule includes a control sequence which directs the synthesis of both the V_L-C_L and V_H-C_H1 polypeptide chains. The control sequence is preferably an inducible promoter such as the lac promoter.

In a further preferred embodiment of the third aspect the polynucleotide molecule includes a sequence encoding a leader peptide which directs the synthesised polypeptide chains to the host cell periplasm. The leader sequence may be the pel B sequence. Preferably, the V_L-C_L and

V_H-C_H1 polypeptide chains associate in the host cell periplasm and are stabilised by one or more disulphide bonds between the chains.

In a further preferred embodiment of the third aspect the heterologous C_H domain sequence is linked to the V_L-C_L sequences or the V_H-C_H1 sequences so that the expressed heterologous C_H domain is attached to the V_L-C_L polypeptide or the V_H-C_H1 polypeptide.

In a further preferred embodiment of the third aspect the non-human C_H1 sequence is absent from the recombinant polynucleotide construct. The heterologous C_H domain sequence may be linked directly to the non-human V_H sequence to give rise to a chimeric non human V_H-human C_H polypeptide chain. This chimeric polypeptide chain may associate with the non-human V_L-C_L polypeptide chain to form a chimeric Fab fragment. It will be appreciated that such a chimeric Fab fragment will possess a specific antigen binding region, and a human constant region which provides a recognition site for class specific anti immunoglobulin antibodies.

The polynucleotide molecules of the second or third aspects of the present invention may be incorporated into plasmids or expression vectors which may then be introduced into suitable bacterial, yeast, insect or mammalian host cells.

Accordingly, in a fourth aspect the present invention provides a vector comprising a polynucleotide according to the second or third aspects of the present invention.

In a fifth aspect the present invention provides a bacterial, yeast, insect or mammalian host cell transformed with a vector according to the fourth aspect of the present invention.

In a sixth aspect the present invention provides a method of producing a chimeric antibody conjugate which comprises culturing a host cell according to the fifth aspect of the present invention under conditions enabling the expression of the conjugate and optionally recovering the conjugate.

In a seventh aspect the present invention provides a chimeric antibody conjugate produced by a method according to the sixth aspect of the present invention.

In yet another aspect of the present invention, the bifunctional molecule is able to bind to antibodies or antibody-like molecules and thereby label them with epitopes from immunoglobulin constant regions derived

from different species. The complex thus formed has the properties of a specific positive antibody control: a ligand binding site with specificity for the antigen, hapten or drug in question and epitopes or domains which are recognised by immunoglobulin binding reagents. The bifunctional molecules of this aspect of the invention may be produced by recombinant DNA technology. Alternatively, recombinant fragments may be linked by conventional chemical coupling technologies.

Accordingly, in an eighth aspect the present invention provides a bifunctional molecule for use in labelling an antibody of a first species, the bifunctional molecule comprising a binding region which binds to the antibody of the first species or to one or more groups provided thereon, and a constant region derived from an antibody of a second species, the constant region comprising at least one C_H domain or an epitope thereof.

The order of the binding and constant regions on the bifunctional polypeptide is not critical. The order may be either (N terminus)—binding region—constant region—(C terminus) or vice versa, ie (N terminus)—constant region — binding region —(C terminus).

In a ninth aspect the present invention provides a complex formed between (i) an antibody or biologically active fragment thereof derived from a first species and (ii) a bifunctional molecule, the bifunctional molecule comprising a binding region which binds to the antibody of the first species or to one or more groups provided thereon, and a constant region derived from an antibody of a second species, the constant region comprising at least one C_H domain or an epitope thereof.

By "biologically active fragment" we mean a fragment which mimics the binding of the antibody derived from the first species to at least one antigenic determinant.

In a preferred embodiment of the eighth and ninth aspects, the binding and constant regions of the bifunctional molecule are separated by a linker molecule. The linker molecule may be a short peptide. Preferably, the linker molecule is a peptide of between 1 and 20 amino acids in length, more preferably between 1 and 10 amino acids in length, and more preferably between 2 and 5 amino acids in length.

In a further preferred embodiment of the eighth and ninth aspects, the binding region is not derived from an antibody. By this we mean that the

binding region is preferably not (i) a Fab fragment, (ii) a portion of a Fab fragment, (iii) an ScFv fragment or (iv) a portion of an ScFv fragment.

In one embodiment of the eighth and ninth aspects, the binding region binds directly to the antibody derived from the first species.

In a further preferred embodiment of the eighth and ninth aspects, the binding region is derived from a protein selected from the group consisting of, *Streptococcal* protein G (described in Björck and Kronvall (1984), and Boyle and Reis (1987), the entire contents of which are incorporated herein by reference) *Staphylococus aureus* protein A (described in Uhlen et al. (1984), and Boyle and Reis (1987), the entire contents of which are incorporated herein by reference) and *Peptostreptococcus magnus* protein L (which is described in Åkerström and Björck (1989), the entire contents of which is incorporated herein by reference). In a further preferred embodiment, the binding region comprises one of the immunoglobulin binding regions of *Staphylococcus aureus* protein A. The immunoglobulin binding region of *Staphylococcus aureus* protein A may be fragment B.

In a further preferred embodiment of the eighth and ninth aspects, the binding region comprises a mouse Fc γ receptor or fragment thereof. The mouse Fc γ receptor may be selected from the group consisting of FcγRI, which specifically binds monomeric mouse IgG2a; FcRII, which binds aggregated IgG1, IgG2a and IgG2b; and FcγRIII, which binds the minor subclass IgG3 (see Heusser et al., 1977; Segal et al., 1978; Unkeless et al., 1988; Hogarth et al., 1987; Kulczycki et al., 1990, the entire contents of which are incorporated herein by reference).

In another preferred embodiment of the eighth and ninth aspects, the binding region comprises a histidine rich glycoprotein (as described in Borza et al., 1996 and Gorgani et al., 1997, the entire contents of which are incorporated herein by reference).

In another embodiment of the eighth and ninth aspects, the binding region binds to one or more groups provided on the antibody of the first species. Preferably, the group(s) is a biotin molecule and the binding region comprises streptavidin (described in Argaraña et al. (1986), US 5672691 and US 5489528, the entire contents of which are incorporated herein by reference) or a fragment thereof.

In a further preferred embodiment of the eighth and ninth aspects of the present invention, the first species is a rat or a mouse.

In a further preferred embodiment of the eighth and ninth aspects, the antibody of the first species is a monoclonal antibody. In a further preferred embodiment, the antibody of the first species is an IgG antibody.

In a further preferred embodiment of the eighth and ninth aspects, the antibody constant region is not a naturally occurring Fc fragment.

In a further preferred embodiment of the eighth and ninth aspects, the antibody constant region comprises or consists of a non-naturally occurring combination of immunoglobulin C_H domains or epitopes thereof. The constant region may include or consist of two C_H domains of the same type, for example, two $C_H3\mu$ domains. Alternatively, the constant region may include or consist of two different domains. The two different domains, or epitopes thereof, may be derived from antibodies of different classes. In a preferred embodiment, the constant region consists of a single C_H domain.

In a further preferred embodiment of the eighth and ninth aspects, the second species is a human. It will be appreciated, however, that the second species may be non-human (for example, bovine, canine, ovine, equine, feline or caprine) in cases where the bifunctional molecule or complex is to be used as a positive control reagent in assays involving sera derived from non-human species.

In a particularly preferred embodiment of the ninth aspect of the present invention, the bifunctional molecule is suitable for combination with mouse IgG as a positive IgM control and the constant region comprises one or more $C_H3\mu$ domains.

In a particularly preferred embodiment of the ninth aspect of the present invention, the bifunctional molecule is bound to a location on the antibody (or fragment thereof) of the first species which does not significantly hinder the binding between the antibody (or fragment thereof) and its specific antigen.

In a further preferred embodiment of the complex according to the ninth aspect, the affinity between the binding region and the antibody or biologically active fragment thereof derived from the first species is sufficient to form a stable complex in solution. Preferably, the binding region has a K_D for the antibody of less than 10^{-6} M. More preferably, the K_D is less than 10^{-8}M and more preferably less than 10^{-9}M.

In a further preferred embodiment of the eighth and ninth aspects, the antibody constant region is modified in order to facilitate the production

of the molecule, or to reduce aggregation of individual bifunctional molecules, without substantially altering the characteristic epitopes of the domain. For example, a cysteine residue usually associated with the formation of an inter-chain disulphide bond may be mutated to serine. In another example, a bifunctional molecule which contains a fragment of *Staphylococcal* protein A linked to a human Cγ3 domain may aggregate because of the high affinity of the protein A fragment for human IgG constant domains. This aggregation may be circumvented by a substitution His to Arg at position 435. Evidence suggests that the lack of binding of protein A to human IgG subclass 3 is related to the substitution of Arg for His at position 435 (see Deisenhofer, 1981, the entire contents of which are incorporated herein by reference).

It will be appreciated by persons skilled in the art that within the context of the present invention, the preferred C_H domains or epitopes will be dependent on the intended use of the bifunctional molecule. For example, if the bifunctional molecule or complex is to be used as a replacement for positive IgM control sera, the preferred C_H domains or epitopes will be C_H μ domains or epitopes. Alternatively, if the bifunctional molecule or complex is to be used as a replacement for positive IgG control sera, the preferred C_H domains or epitopes will be a C_H γ domains or epitopes. If the bifunctional molecule or complex is to be used as a replacement for positive IgA control sera, the preferred C_H domains or epitopes will be a C_H α domains or epitopes.

In a tenth aspect, the present invention provides an isolated polynucleotide encoding a bifunctional molecule according to the eighth aspect of the present invention.

The polynucleotide molecule of the tenth aspect of the present invention may be incorporated into plasmids or expression vectors which may then be introduced into suitable bacterial, yeast, insect or mammalian host cells.

Accordingly, in en eleventh aspect the present invention provides a vector comprising a polynucleotide according to the tenth aspect of the present invention.

In a twelfth aspect the present invention provides a bacterial, yeast, insect or mammalian host cell transformed with a vector according to the eleventh aspect of the present invention.

In a thirteenth aspect the present invention provides a method of producing a bifunctional molecule which comprises culturing a host cell according to the twelfth aspect of the present invention under conditions enabling the expression of the bifunctional molecule and optionally recovering the bifunctional molecule.

In a fourteenth aspect the present invention provides a bifunctional molecule produced by a method according to the thirteenth aspect of the present invention.

In a fifteenth aspect the present invention provides a method of producing a complex according to the ninth aspect which comprises admixing an antibody or biologically active fragment thereof derived from a first species with a bifunctional molecule according to the eighth aspect of the present invention.

Methods for detecting antibodies in biological samples are well known. In general, these methods involve incubation of the sample with (i) a antigenic determinant characteristic of a particular disease, and (ii) an anti human Ig antibody. The antibody measurement is generally compared to a control measurement obtained by incubating the antigenic determinant characteristic of the disease and the anti human Ig antibody with a positive control serum obtained from an individual with the disease. The present inventors have found that the chimeric antibody conjugates of the present invention react in diagnostic tests in a manner similar to class specific positive control serum.

Accordingly, in a sixteenth aspect the present invention provides a method for detecting an antibody in a biological sample which involves comparing the level of detection obtained with the biological sample to the level of detection obtained with a positive control, wherein the positive control comprises a chimeric antibody conjugate according to the first aspect, or a complex according to the ninth aspect.

In a preferred embodiment of the sixteenth aspect of the present invention, the biological sample is a human biological sample.

In a further preferred embodiment of the sixteenth aspect of the present invention the antibodies to be detected are antibodies characteristic of a disease selected from but not limited to dengue fever, Japanese encephalitis, rubella, spotted fever, herpes infection, parvovirus infections, melioidosis, typhoid, leptospirosis, malaria, yellow fever, whooping cough,

systemic candidiasis/thrush, chicken pox, shingles, AIDS, hepatitis, liver cancer, cervical cancer, infectious mononucleosis, nasopharyngeal carcinoma, Ross River fever, brucella, exanthum subitum (Sixth disease/Roseola infantum), erythema ingfectiosum (Fifth disease), Q Fever, cold sores, genital herpes, spotted fever, scrub typhus.

The antibody to be detected in the biological sample may be an antibody of any class. In a preferred embodiment, however, the antibody is an IgM antibody.

The terms “comprise”, “comprises” and “comprising” as used throughout the specification are intended to refer to the inclusion of a stated component or feature or group of components or features with or without the inclusion of a further component or feature or group of components or features.

The invention will now be described in detail by reference to the following non-limiting Figures and Examples.

Brief Description of the Figures

Figure 1 shows the structure of intact IgG antibody (a) along with the two sub fragments capable of being produced in prokaryotic or lower eukaryotic cells, Fab (b) and scFv (c). The antigen binding region, the location of the CDR loops are indicated.

Figure 2 illustrates one embodiment of the invention. The single polypeptide chain protein folds into two domains. The scFv region derived from mouse DNA sequences folds to form the specific antigen binding site. The C-domain derived from human DNA sequences of immunoglobulin constant regions folds to provide binding epitopes for heterologous, class specific anti human immunoglobulin sera.

Figure 3 shows in cartoon form the two regions of the bifunctional binding molecule and illustrates one embodiment of the invention. Also shown is the complex formed between the bifunctional molecule and intact mouse IgG in which the mouse IgG is decorated with antibody C domains, preferably from human immunoglobulin heavy chains.

Figure 4 shows a further embodiment of the invention in which a complex is formed between the bifunctional molecule and one or more groups introduced onto the antibody of species A. In the embodiment

illustrated, the group is biotin, the first region which binds to the group is streptavidin or a fragment thereof and the antibody is mouse IgG.

Figure 5 illustrates in cartoon form the structure of the bacterial expression plasmid containing the sequence of one embodiment of the invention. The expression cassette contains a chemically inducible promoter, lac, followed by sequence encoding the components of the chimeric protein. The pel B sequence directs the synthesised protein to the cell periplasm, the V_H and V_L regions joined by linker sequence are the mouse scFv antibody, and C_H is the human immunoglobulin constant domain sequence inserted between the Not I and Sac II sites. FLAG is an octapeptide recognition sequence recognised by the antibody anti FLAG® M2 (Eastman Kodak Co., New Haven, CT) used for monitoring expression; stop designates a stop codon and TT designates a transcription terminator. The remainder of the vector is derived from vector pUC19 (Yanisch-Perron et al., 1985). The entire vector minus the specific inserts is denoted pGC (Coia et al., 1996)

Figure 6 illustrates the reactivity in ELISA of the 4 different human IgM C_H domain constructs linked to the scFv 1C3 (anti-glycophorin). With glycophorin bound to the ELISA well, samples containing the gene product were introduced and incubated for 1 hour at RT. After extensive washing, wells were probed with polyclonal anti human IgM antiserum raised in sheep and labelled with horseradish peroxidase. After 1 hour incubation at RT and extensive washing, ABTS was added for colour development which was read at 405 nm after 20 mins.

Figure 7 illustrates the reactivity in a dengue IgM capture ELISA of unfractionated periplasm taken from an expression culture of the 13C11(anti dengue)scFv-$C_H3\mu$ domain construct. Positive, negative and calibrator controls were supplied with the test kit (see text) and used as directed: (100 μl per assay) at a dilution of 1/100 in serum diluent. Other reagents were as supplied with the kit. Periplasm was diluted 1/5, 1/10, 1/50 and 1/100. 100 μl of each dilution including undiluted periplasm were added to test wells on the ELISA plate, covered and incubated for 1 hour at 37°C. After 4 washes with diluted wash buffer, 100 μl of a combined mixture of stablized dengue virus antigens with peroxidase labelled anti-dengue monoclonal antibody were added and the plate further incubated for 1 hour at 37°C. After 6 washes with diluted wash buffer, 100 μl TMB solution was added and colour

development proceeded for 10 mins. 100 μl Stop solution was then added and the colour read at 450nm.

Negative, Calibrator and Positive refer to serum controls supplied with the test. Peri, peri 1/5-peri 1/100 refer to test samples containing the 13C11(anti dengue)scFv- $C_H3\mu$ domain construct. Peri neg control is derived from a culture of 1C3(antiglycophorin)- $C_H3\mu$ domain chimera (Example 1).

Figure 8 shows the DNA sequence of the expression cassette (from pGC vector) containing fragment B from *Staphylococcus aureus* Protein A joined via a short linker to the human IgM heavy chain $C_H3\mu$ domain, together with a terminal FLAG® tag sequence, pel B leader and trp A terminator.

Figure 9 shows in cartoon form the constituents of the ELISA used to demonstrate the binding of various mouse IgG subclasses to the bifunctional linking reagent described in Example 1 which has bound to immobilised polyclonal anti human IgM antibody, raised in sheep.

Figure 10 shows the verified sequence of an expression cassette in pGC comprising the pel B leader sequence, core streptavidin, human IgM C_H3 domain and FLAG® tag.

Figure 11 shows results from size exclusion chromatography on Superdex200 of a sample containing refolded Streptavidin-$C_H3\mu$ in phosphate buffered saline. Flow rate was 0.5ml/min.

Detailed Disclosure of The Invention

In one aspect the present invention relates to a recombinant chimeric antibody molecule. One region of this chimeric molecule comprises an antigen binding region derived from an antibody.

Fragments of antibody molecules containing predominantly antigen binding regions have been synthesized using prokaryote or lower eukaryote expression systems (eg bacterial or yeast cells) (see, for example, PCT/AU93/00491, the entire disclosure of which is incorporated herein by reference). The antigen binding site is composed of amino acid residues formed in up to six surface loops at the extremity of the molecule. The loops on the outer domain are termed complementarity determining regions (CDRs) and provide the specificity of binding of the antibody to the antigenic target, by variation in the amino acid composition of these surface loops. The antigen binding regions of both intact IgG and sub-fragments are illustrated in Figure 1.

In order to stabilise the paired associations of the V_H and V_L regions produced by such cultures, these regions may be expressed as one continuous polypeptide chain where there is a region of amino acids of sufficient length and flexibility interspersed between the C-terminus of one domain and the N-terminus of the other to allow the two domains to dock correctly with each other to correctly position the CDR loops. Methods of manufacture of covalently linked single chain Fv fragments are disclosed in US-4,946,778, US-5,132,405 and WO 94/07921 the entire contents of which are incorporated herein by reference.

Alternatively, the antigen binding domains can be produced as Fab fragments where two polypeptide chains V_H - C_H1 and V_L - C_L are synthesised separately from mouse gene sequences and the subsequent formation of heavy and light chain fragment pairs are stabilised by a disulphide bond between the two chains. (See, for example, Better et al., 1988, Skerra, 1993, Dolezal et al., 1995, the entire contents of which are incorporated herein by reference). A preferred source of paired V_H and V_L genes for the formation of antigen binding domains is cDNA prepared from mRNA isolated from mouse monoclonal antibody cell lines.

In one preferred embodiment the chimeric antibody conjugate is a polypeptide chain which, when expressed in *E. coli*, yeast or mammalian cells from a single gene construction, folds to produce two separate functional domains, as shown in Figure 2. The first domain binds a specific antigen, and the second domain contains a specific immunoglobulin constant domain sequence (epitope) which may be recognised and bound by antibodies, prepared in rabbits, sheep or other such animal, by immunisation with class specific human immunoglobulins.

The first functional domain may consist of an antigen binding domain, formed by paired antibody V_H and V_L regions either a) linked in active conformation via a flexible peptide linker as in a scFv molecule or b) linked to mouse C_H and C_L domains as in a Fab antibody fragment. The flexible linker used to link the V_H and V_L regions as in a scFv molecule may be a polypeptide of between 12 and 30 amino acids in length (Huston *et al.*, 1991). The V_H and V_L gene sequences which code for the antibody V_H and V_L regions may be amplified via PCR from cDNA of non-human origin (usually prepared from a mouse monoclonal antibody cell line producing antibody with binding specificity for the antigen being assayed in the diagnostic test in

question). Any antigen binding specificity may be incorporated in this domain in either the Fab or scFv conformation. Preferably, a mouse monoclonal antibody cell line exists with that specificity or a V_H /V_L pair has been selected from an antibody phage library with binding specificity for that particular antigen.

The second functional domain may consist of either a single heavy chain constant domain or several in tandem which display binding sites (epitopes) for class specific polyclonal anti immunoglobulin antisera, also known as capture antibodies. The C_H region gene sequences may be amplified via PCR from cDNA prepared from mRNA isolated from peripheral blood lymphocytes. The C_H regions can be from any of the immunoglobulin heavy chain genes, (those for IgM, IgG, IgA, IgD, IgE) and the gene product from the particular C_H region is bound by the class specific anti immunoglobulin antiserum.

It will be appreciated that chimeric antibody conjugates of the present invention are capable of reacting in diagnostic tests in a manner similar to a class specific positive control serum. The chimeric conjugate will bind to a specific antigen, and will in turn be bound by the class specific capture antibodies which determine its immunoglobulin. An advantage of the conjugates of the present invention is that they may be produced in large quantities, free of contaminants, by recombinant DNA technology.

In yet another aspect the present invention relates to a bifunctional molecule which is able to bind to antibodies or antibody-like molecules and thereby label them with epitopes from immunoglobulin constant regions derived from different species. Preferably, the bifunctional molecule is a single polypeptide chain which when expressed in *E. coli*, yeast or mammalian cells folds to produce two separate functional domains, as shown in Figure 3. The first domain preferably binds to a specific region of an antibody, for example, mouse IgG, and the second domain contains a specific immunoglobulin constant domain sequence (epitope) which may be recognised and bound by antibodies, prepared in rabbits, sheep or other such animal, by immunisation with class specific human immunoglobulins.

The affinity of binding between the binding region and antibody is preferably sufficient to form a stable complex in solution between mouse IgG and the bifunctional molecule. The region on the antibody to which the bifunctional molecule binds is preferably in a location which will not

sterically hinder the binding between the mouse IgG antibody binding site and its specific antigen.

In one embodiment, the binding region binds to a group provided on the antibody. This particular embodiment is illustrated in Figure 4, in which the binding region comprises streptavidin or a fragment thereof and the antibody is mouse IgG.

The invention will be described in detail by reference to the following non-limiting examples.

EXAMPLE 1

Production of a C-domain (IgM) extended scFv

The gene sequences of the four constant domains (C domains) of human IgM heavy chain were separately amplified from cDNA prepared from mRNA isolated from human peripheral blood lymphocytes using polymerase chain reaction techniques. The design of the oligonucleotide primers used in the amplifications was based upon the 5' and 3' base sequence of each of the four IgM heavy chain exons, obtained through GENBANK accession X14940 (Dorai and Gillies, 1989).

In the primers, specific restriction enzyme recognition sequences were added (*Not*I at the 5' end and *Sac*II at the 3' end) to each exon sequence to facilitate the introduction of the C domain sequence at a specific site in a previously constructed plasmid expression vector. The expression cassette of this *E. coli* plasmid vector (pGC; Coia et al., 1996) contained V_H and V_L sequences from the mouse monoclonal antibody cell line 1C3, (Rylatt *et al.*, 1990, WO91/04492) with binding specificity for human glycophorin. These were arrayed in the scFv format, with the 3' end of the V_H sequence linked to the V_L sequence via a 45 bp sequence which coded for the protein sequence GGGGSGGGGSGGGGS. In the synthesized protein, this flexible linker region allows the correct docking of V_H with V_L. The site for the introduction of the heavy chain exon was at the 3' end of the mouse V_L sequence without any intervening sequence, save that for the restriction sites, as shown in Figure 5. Fragments were ligated together using the normal protocols and the ligation mix used to transform *E. coli* strain XL1-Blue by electroporation.

Recombinant protein was produced from positively transformed colonies of the four different constructions (each with a different IgM C-domain sequence) by induction of the plasmid lac promoter with 0.5 mM

IPTG in log phase cultures grown at 30°C. After a further 4 hours incubation at 25°C, the cell pellet was harvested by centrifugation, and the contents of the cell periplasm isolated using the protocol of Minsky et al. (1986).

The periplasmic fraction was assayed by ELISA for the presence of protein molecules with the following properties - 1) the ability to bind to immobilised glycophorin on the ELISA plate and 2) a target for the binding of horseradish peroxidase-labelled polyclonal anti human IgM serum (prepared in sheep, Silenus Laboratories, Melbourne Australia). The results in Figure 6 show that the construction with IgM constant domain 3 ($C_H3\mu$) gave the strongest reaction with the labelled polyclonal antiserum to human IgM. This was followed by domain $C_H2\mu$, with domains $C_H4\mu$ and $C_H1\mu$ showing the weakest reactions. It was a surprising observation that the majority of the reactivity of the polyclonal antiserum was directed to one domain of the IgM heavy chain. Thus, for this particular polyclonal antiserum directed against human IgM, the construct scFv-$C_H3\mu$ is a molecule with the preferred properties of a substitute for a positive human serum control.

A scFv with binding specificity for human glycophorin was solely used to demonstrate the present invention. As will be appreciated by persons skilled in this field, the antigen binding portion of the conjugate may be replaced with binding specificities to other antigenic entities which are the focus of a wide range of diagnostic test applications.

EXAMPLE 2

Construction of extended scFv (13C11 antidengue) linked to a human IgM C domain

The reagent was produced from a DNA construct in which the coding region for a mouse scFv directed against dengue virus was genetically linked to that of the third constant domain of human IgM heavy chain ($C_H3\mu$). cDNA was prepared from mRNA isolated from the mouse monoclonal antibody cell line 13C11, which specificity for Dengue virus surface antigens (Queensland University of Technology and PanBio Pty Ltd.). Immunoglobulin V_H and V_L domain DNA sequences were amplified from the cDNA using polymerase chain reaction and oligonucleotide primer sets according to Zhou et al. (1994). These were linked in the scFv format V_H - linker- V_L, where the linker was a 45 bp nucleic acid sequence coding for the protein sequence GGGGSGGGGSGGGGSGGGGS. The resultant fragment was

digested with restriction endonucleases *Nco* I and *Not* I and purified by agarose gel electrophoresis. The expression vector as described in Example 1 which contained the 1C3 (antiglycophorin) scFv- $C_H3\mu$ domain sequence was also digested with *Nco* I and *Not* I to excise the coding sequence for the 1C3 scFv. The remainder of the vector (plus C domain coding sequence) was purified and ligated with the digested 13C11 (anti dengue) scFv coding region using standard protocols. This ligated DNA was then used to transform *E. coli* strain XL1-Blue by electroporation. Positive colonies were verified both by DNA sequencing and small scale protein expression.

E. coli strain TOPP6 (Stratagene, La Jolla, CA) was transformed with purified plasmid DNA from a verified clone and used for protein expression. 500 ml of 2xYT medium (1.0 % Yeast extract, 1.6% Bacto Tryptone, 1.0 % NaCl) supplemented with ampicillin at 200 μg/ml was inoculated with an overnight culture of the transformed TOPP6 cells and incubated at 37°C with agitation until the A_{600} had reached 1.0. IPTG (isopropylthiogalactoside) was added to a concentration of 0.5 mM to induce the expression of the chimeric gene construct. The culture was shaken for a further 4 hours at a 25°C. Cells were harvested by centrifugation and the periplasmic contents isolated using the protocol of Minsky et al. (1986).

Dilutions of the unfractionated periplasm were analysed in the Dengue IgM Capture ELISA kit (PanBio Ltd, Windsor, QLD, Australia: Sang et al., 1998) using positive, negative and calibrator serum controls as supplied in the kit. In the assay, human IgM antibodies are captured by surface bound polyclonal anti human IgM antiserum (Silenus), and incubated with soluble dengue antigens plus a peroxidase labelled, dengue antigen-specific monoclonal antibody, to reveal the presence of dengue specific antibodies.

The results are shown in Figure 7. The periplasm fractions reacted positively, with the neat, 1/5 and 1/10 dilutions giving higher absorbance readings at 450 nm than the normal control. At 1/50, the periplasm still gave an absorbance reading greater than the calibrator control, which marks the cut-off between positive and negative reactions. It was calculated that an absorbance reading equivalent to the positive control would have been obtained from a periplasm dilution of 1/30. Periplasm from a culture of an unrelated chimera (1C3-$C_H3\mu$, anti-glycophorin, see Example 1) showed no positive reaction in this test.

EXAMPLE 3

Construction of Extended scFv (13C11 anti dengue) linked to a human IgG C-domain

The gene sequences of human IgG constant domains 2 and 3 were separately amplified from cDNA from mRNA isolated from human peripheral blood lymphocytes using polymerase chain reaction techniques. The design of the oligonucleotide primers used in the amplifications were based upon the 5´ and 3´ sequences for each of the heavy chain exons, obtained through Genbank accession no E06998.

Sequences coding for *Not*I and *Sac*II restriction sites were added to the 5´ and 3´ end respectively of the $C_H2\gamma$ and $C_H3\gamma$ sequences to enable the insertion into pGC 13C11-$C_H3\mu$ from which the $C_H3\mu$ sequence had been removed as a *Not*I—*Sac*II fragment.

Expression in *E. coli* and purification of product was performed as described in Example 2. The presence of product in the periplasmic fraction was confirmed by analysis of samples by polyacrylamide gel electrophoresis and Western blotting, probing the FLAG® tag using mouse anti FLAG® M2 antibody (Hopp et al., 1988) The products were denoted 13C11$C_H2\gamma$ and 13C11$C_H3\gamma$ respectively.

PanBio Indirect Dengue ELISA using 13C11CH2γ and 13C11CH3γ

Periplasmic samples containing 13C11$C_H2\gamma$ and 13C11CH3γ were diluted 1/10 in PBS /0.05% Tween 20 and 100 μl loaded in duplicate on an ELISA plate coated with dengue antigens (PanBio Dengue Indirect ELISA Kit Cat DET500) and incubated for 1 hour at 37°C. Controls included PBS/Tween 20 as negative control and positive IgG control serum which was probed with both anti human IgM and IgG antibodies. After 6 washes with PBS/0.05% Tween20, samples and controls were then probed with either sheep anti human IgM or IgG labelled with horseradish peroxidase (Silenus/AMRAD, Melbourne) at 1:1000 dilution in PBS/0.05% Tween20. The plate was incubated at 37°C for 1 hour then washed 6 times as previously. 100μl TMB reagent, supplied with the kit, was added to each well, the plate incubated at room temperature for 10 min, then the reaction was stopped by the addition of 100μl 1M phosphoric acid. Colour intensity was read at 450 nm. The results are shown in Table 1.

Table 1

	Absorbance 450nm	
	HRP-anti Human IgG	HRP-anti Human IgM
13C11 CH3γ Periplasm 1/10	1.684, 1.670	0.111, 0.109
13C11 CH2γ Periplasm 1/10	0.500, 0.586	0.133, 0.113
Human IgG (Dengue Positive) 1/100	0.971, 0.940	0.202, 0.361
PBS/0.05% Tween 20	0.308, 0.261	0.112, 0.275

Both 13C11C_H3 γ and 13C11C_H2γ extended scFvs show a positive reaction above background in this ELISA, the results with the 13C11 C_H3γ periplasm being comparable or better than the Human IgG dengue positive control. The reaction with the construct containing the human IgG C_H3γ domain gave a stronger response than with the CH2γ domain, indicating that extended scFvs with a C_H3γ domain would be the preferred construct for use as a replacement IgG positive control.

EXAMPLE 4

Production of a bifunctional molecule containing the B fragment of Staphylococcal Protein A linked to a human IgM C-domain (FB-$C_H3\mu$)

The gene sequence for fragment B of Protein A (FB) from *Staphylococcus aureus* was amplified from chromosomal DNA prepared from strain ATCC 25923 using polymerase chain reaction techniques. The design of the oligonucleotide primers used in the amplification was based upon the 5' and 3' base sequences as reported by Uhlen et al. (1984), also GENBANK accession J01786. In the primers specific restriction enzyme recognition sites were added *Nco*I at the 5' end and *Not*I at the 3' end to facilitate the introduction at a specific site in a previously constructed plasmid expression vector (pGC; Coia et al., 1996).

In this vector the sequence encoding a human IgM C domain ($C_H3\mu$) had previously been inserted as a *Not*I - *Sac*II fragment. A short sequence encoding the three amino acids Ser, Asp, Pro was included downstream of the FB fragment and before the *Not I* site to introduce some flexibility between the FB domain and the human $C_H3\mu$ domain. The Human $C_H3\mu$ domain had previously been amplified from cDNA prepared from mRNA

isolated from human peripheral blood lymphocytes using polymerase chain reaction techniques, using oligonucleotides based upon the 5' and 3' sequences of the domain obtained through GENBANK accession X14940 (Dorai and Gillies, 1989). We have demonstrated herein that human IgM C domain 3 ($C_H3\mu$) contains the major reactive epitopes which are bound by several polyclonal and monoclonal anti human IgM antisera capture reagents. Fragments were ligated together using standard ligation protocols and the ligation mix then used to transform *E. coli* strain XL1 Blue by electroporation. The complete DNA sequence of the expression cassette comprising the pel B leader sequence, fragment B of *S. aureus* Protein A, human $C_H3\mu$ domain, and FLAG® (a tag recognition sequence, Hopp et al., 1988) was verified by using automatic DNA sequencing methods and is shown in SEQ ID NO: 2 and Figure 8.

Recombinant protein was produced from positively transformed *E. coli* colonies by induction of the lac promoter with 0.2mM IPTG (isopropyl β-d-thio galactoside) in log phase cultures grown at 37°C. Cultures were induced at a A_{600} of 1.5 - 2 and incubated for a further 16 hours at 18°C. The cell pellet was then harvested by centrifugation and the contents of the cell periplasm isolated using the protocol of Minsky et al. (1986).

Analysis of the periplasmic fraction by polyacrylamide gel electrophoresis and Western blot probed with mouse anti FLAG® M2 antibody revealed the presence of a FLAG-tagged component in the periplasm with an approximate molecular weight (Mr) of 20 kD. The periplasmic fraction was then assayed by ELISA to reveal the presence of protein molecules with the following properties:

1. The ability to bind to polyclonal anti human IgM antibody prepared in sheep and immobilised on the ELISA.
2. The ability to bind to intact mouse IgG as detected by the addition of goat anti mouse IgG antibody, labelled with horseradish peroxidase which reacts with TMB (3',3',5',5',-tetramethylbenzidine) to produce a coloured product measured at 450 nm.

Property 1 was demonstrated by an ELISA in which crude periplasm was reacted with immobilised polyclonal anti human IgM capture antibody, then probed with mouse anti FLAG® antibody together with goat anti mouse IgG labelled with horseradish peroxidase to detect the C terminal FLAG tag. ELISA plate wells were coated with polyclonal sheep anti human IgM

antiserum (Sang et al., 1998), blocked with 5% Skim milk powder in PBS at 37°C for 1.5 hours. Between each addition step, the wells were washed 10 times with PBS-0.05% Tween 20. Each addition (100 μl) was incubated for 20 min at room temperature. Mouse anti FLAG® (Eastman Kodak Co, New Haven, CT) was used at 1ug/ml in PBS-0.05% Tween 20. Goat anti mouse IgG Fc-HRP was used at 0.16 ug/ml in PBS-0.05% Tween 20. Colour was developed by the addition of 100 μl TMB reagent (3',3',5',5',-tetramethylbenzidine plus H_2O_2), incubation at room temperature for 10 mins followed by the addition of 100 μl 1M Phosphoric acid, and incubation at room temperature for 10 mins. Wells were then read at 450 nm in an ELISA micro plate reader. The results are shown in Table 2.

Table 2

1st addition	2nd addition	3rd addition	A450
Periplasm	mouse anti FLAG®	Goat anti-mouse Ig HRP	>3.000 (4 wells)
PBS	mouse anti FLAG®	Goat anti-mouse Ig HRP	0.124 ± 0.008 (3 wells)
PBS	PBS	Goat anti-mouse Ig HRP	0.094

The positive result could arise from a combination of the binding of anti FLAG via the FLAG epitope, or the binding of the mouse IgG with the FB domain on the bifunctional molecule. Regardless of the proportional contributions from either of these reactions, the result demonstrates that the bifunctional molecule can be captured by anti human IgM capture antibodies.

Property 2 was tested using an ELISA sandwich as shown in Figure 9. Four mouse IgG subclasses were each individually tested for their ability to bind to the bifunctional molecule. Reagents were from AMRAD, Melbourne, Australia (Mouse IgG1: 12CONT01 batch WD12A; IgG2a: 12CONT02 batch UI17A; IgG2b: 12HHLA01 batch UK18A; IgG3 Rota Ser4 batch UK07-B1). Each was diluted to 1 μg/ml with PBS/0.05% Tween20 before use. The control linker reagent FB-$C_H3\mu$ was diluted serially from 1/20 to 1/320 and 100μl loaded into ELISA wells coated with stabilised sheep anti-human IgM

(PanBio Pty Ltd) and incubated for 1 hour at 37°C. After 6 washes with PBS/0.05% Tween20 100μl of each diluted mouse IgG subclass was added and the plate incubated a further hour at 37°C. After 6 washes in PBS/0.05% Tween20, 100μl HRP-labelled goat anti mouse IgG Fc (Pierce Chemical Co. Rockford, Ill) was added at 0.16μg/ml, the plate then incubated for 1 hour and washed 6 times. The reaction was developed with 100μl TMB solution for 10 min, stopped by adding 100 μl 1M phosphoric acid and the absorbance read at 450nm. The results are shown in Table 3.

Table 3

Dilution of Control linker reagent FBCH3μ	Mouse IgG1 1 μg/ml	Mouse IgG 2a 1 μg/ml	Mouse IgG 2b 1 μg/ml	Mouse IgG3 1 μg/ml
1/20	2.425	1.134	0.762	0.599
1/40	2.007	0.555	0.787	0.489
1/80	2.010	0.776	0.578	0.289
1/160	1.429	0.581	0.399	0.373
1/320	1.123	0.320	0.309	0.302
PBS	0.260 (av of 3)			

These results show that under the conditions of the reaction, the binding of mouse IgG subclasses to the control linker reagent is ranked in the following order: IgG1, IgG2a, IgG2b, IgG3, from highest to lowest. Control reagents formed using a Staphylococcus protein A fragment B-C domain linker would be most successful if mouse IgG1 is used to form the complex. It will be appreciated by those skilled in the art that if the subclass of the mouse monoclonal antibody is IgG3, a front end domain other than protein A would preferably be used to produce the bifunctional molecule. Suitable alternatives are described in the "Summary of the Invention" section of this specification..

The bifunctional molecule was separated from other periplasmic components by affinity chromatography on matrix bound mouse anti FLAG® antibody. The fraction which bound to the column was eluted with 0.1 M Glycine HCl pH 3.0 then adjusted to neutrality with saturated Tris.

The bifunctional molecule (denoted FB-$C_H3\mu$) was concentrated to a final concentration of approx 1.2 mg/ml and used in an indirect ELISA test.

Human Herpes Virus 6 (HHV6) Indirect ELISA

Tissue culture supernatant containing mouse monoclonal antibody to HHV6 was diluted 1/50 in PBS-0.05%Tween 20 and added to ELISA plates previously coated with HHV6 antigen and incubated at 37°C for 30 min. After 4 washes with PBS-Tween, FB-$C_H3\mu$ was added to subsequent wells in doubling dilutions from 1/20 to 1/1280 in similar diluent and incubated a further 30 mins at 37°C. After 4 washes with diluent, polyclonal sheep anti human IgM labelled with horseradish peroxidase (AMRAD, Melbourne, 1/1500) was added and incubated 20 min at 37°C. Wells were washed 6 times with PBS and the peroxidase reaction was developed using 100 µl TMB solution (3', 3', 5', 5'-Tetramethylbenzidine; BioChem ImmunoSystems Italia SPA) for 10 mins and the reaction stopped by the addition of 100 µl 1M Phosphoric acid. Results are presented in Table 4. The results demonstrate an effective positive reaction to dilutions as great as 1/80.

Table 4

Dilution of FB-$C_H3\ \mu$	A_{450}
No FB-$C_H3\ \mu$ (zero)	0.050
1/20	0.771
1/40	0.512
1/80	0.384
1/160	0.197
1/320	0.139
1/640	0.087
1/1280	0.079

Use of FB-$C_H3\mu$ control linker in AMRAD Hepatitis E antibody indirect ELISA

The control linker FB-$C_H3\mu$ was mixed with mouse IgG1 monoclonal antibody to the conformational epitope of Hepatitis E virus (Ref code 2E2) and used in an indirect ELISA test, comparing the response to positive and negative serum controls provided with the test kit (AMRAD, Melbourne, Vic).

The control linker sample was partially purified and concentrated from material located in the periplasmic fraction. The control linker and mouse HEV antibody were mixed prior to the assay such that there was a dilution series of mouse monoclonal antibody from 0 to 50 µg/ml at control reagent dilutions of 1:10 and 1:50. The human positive control was serially diluted from 1/200 and the negative control diluted 1/200 with serum diluent supplied with the kit. Samples were added to an ELISA plate (AMRAD hepatitis E virus coated plates batch #1401H037) and incubated at room temperature for 30 min. After 3 washes with PBS/0.05% Tween20, 100µl anti-human IgM–HRP conjugate (Silenus; 1:10,000) was added, incubated a further 30 min at room temperature, washed 3 times and TMB substrate added. After 10 min incubation, the reaction was stopped with 1M sulphuric acid and the plate read at 450nm. The results are shown in Table 5.

Table 5

2E2 Mab conc (µg/ml)	Control reagent Dilution		Positive Control Dilution Series	
	1:10	1:50	Dilution	A450
50	2.92	1.828	1/200	2.745
25	2.931	1.795	1/400	2.135
10	2.880	1.772	1/800	1.525
5	2.900	1.703	1/1600	0.799
2	2.378	1.312	1/3200	0.505
1	2.112	0.792	1/6400	0.296
0	0.025	0.023	1/12800	0.168
			Negative Control 1/200	0.103

These results show that Premixed FB-$C_H3\mu$ control linker/ mouse monoclonal antibody can serve as a suitable positive IgM control in the AMRAD HEV ELISA assay.

Levels which give comparable A450 to serum controls are:

Control Linker 1/10 + Mab 2 µg/ml

Control Linker 1/50 + Mab 50 µg/ml.

No significant background problems are observed indicating that this is a viable option to serum controls in the HEV assay.

EXAMPLE 5

Production of a bifunctional molecule containing the B fragment of Staphylococcal Protein A linked to a human IgG C-domain

The gene sequences of human IgG constant domains 2 and 3 were separately amplified from cDNA prepared from mRNA isolated from human peripheral blood lymphocytes using polymerase chain reaction techniques. The design of the oligonucleotide primers used in the amplifications was based upon the 5' and 3' sequences for each of the heavy chain exons, obtained through Genbank accession no E06998.

Whereas *Staphylococcal* protein A (SPA) exhibits a stronger affinity for human IgG1, 2 and 4 than for mouse IgG subclasses, binding to human IgG3 is negligible (Reis et al,1984). It has been suggested that the substitution of histidine with arginine at position 435 in IgG3 prevents the binding to Protein A (Deisenhofer, 1981). Therefore in order to minimise any self aggregation of a bifunctional construct between Fragment B of SPA and human IgG C domains, it would be preferable to have any C3γ domain sequence contain the IgG3 mutation, Arg^{435}. It is not possible to selectively amplify IgG3 constant region sequences from cDNA because of the close homology of the 5' and 3' terminal sequences between all human IgG subclasses. Consequently the mutation was performed subsequent to the amplification and cloning using the QuikChange™ Site Directed mutagenesis kit (Stratagene, La Jolla, CA).

Sequences coding for *Not*I and *Sac*II sites were added to the 5' and 3' end respectively of $C_H2\gamma$ and $C_H3\gamma$ sequences to enable insertion into the expression vector pGC FB-$C_H3\mu$, shown in Figure 8, from which the $C_H3\mu$ sequence was removed as a *Not*I—*Sac*II fragment.

Expression in *E. coli* and purification of product was performed as described in Example 4.

PanBio IgG Indirect Dengue ELISA using FB-$C_H2\gamma$ and FB-$C_H3\gamma$ control linkers

Test samples were unfractionated periplasmic fractions containing FB-$C_H2\gamma$ and FB-$C_H3\gamma$ control linkers from 500 ml expression cultures. Samples were used neat or diluted 1:10 in PBS/0.05% Tween 20.

Mouse anti dengue monoclonal antibody was clone 13C11 (IgG2a) obtained from PanBio Ltd (Windsor, Qld) at 1.6 mg/ml and used at a final concentration of 1.6 μg/ml diluted in PBS/0.05% Tween 20.

The human positive serum control containing anti dengue IgG antibodies was obtained from PanBio Ltd and is identical to what is supplied in their commercial dengue ELISA test. It was used at a dilution of 1:100 in PBS/0.05% Tween 20.

HRP-labelled sheep anti human IgG (lot TJ19B) was from Silenus/AMRAD (Melbourne) and used at a dilution of 1:1000 in PBS/0.05% Tween 20.

The ELISA plate coated with dengue antigens was as supplied by PanBio in their commercial Dengue ELISA test. It was used without further blocking. All incubations were for 1 hour at 37°C followed by 3x2min washes with PBS/0.05% Tween 20.

The first layer of the ELISA contained 100 μl 13C11 mouse anti dengue monoclonal antibody; control wells contained PBS/0.05% Tween 20. Following incubation and washing as described the samples containing FB-$C_H2\gamma$ and FB-$C_H3\gamma$ were added. Controls contained either human anti dengue IgG serum 1:100 or PBS/0.05% Tween 20. Following incubation and washing, HRP-labelled sheep anti human IgG 1:1000 was added. After incubation and washing, 100 μl TMB solution (containing H_2O_2) was added and incubated for 10 min at room temperature to develop the colour reaction. 100μl 1M phosphoric acid was added to stop the reaction and the plate read in a micro plate reader at 450nm. Results are shown in Table 6.

Table 6

	A450	
13C11mAb + FB- $C_H3\gamma$ periplasm	1.475	1.220
PBS-Tween + FB-$C_H3\gamma$ periplasm (control)	0.564	
13C11mAb + FB-$C_H3\gamma$ periplasm 1:10	0.901	0.825
PBS-Tween + FB-$C_H3\gamma$ periplasm 1:10 (control)	0.268	
13C11mAb + FB-$C_H2\gamma$ periplasm	0.856	0.814
PBS-Tween + FB-$C_H2\gamma$ periplasm (control)	0.411	
13C11mAb + FB-$C_H2\gamma$ periplasm 1:10	0.545	0.521
PBS-Tween + FB-$C_H2\gamma$ periplasm 1:10 (control)	0.279	
PBS-Tween + human anti dengue IgG positive control 1:100	0.930	0.922
PBS-Tween + PBS-Tween	0.276	0.265

Both periplasmic fractions containing FB-$C_H2\gamma$ and FB-$C_H3\gamma$ provide positive reactions in this ELISA when linked with the mouse anti dengue mAb, 13C11, compared to controls. The linker containing the $C_H3\gamma$ domain is the preferred construct to mix with a specific mouse monoclonal antibody to use as a replacement IgG positive control reagent.

EXAMPLE 6

Bifunctional construct using core streptavidin as the Ig binding domain

The protein streptavidin produced by *Streptomyces sp.* has an affinity (K_D) for biotin of the order of 10^{-15} M (Green, 1975; Pähler et al., 1987) Commercially produced streptavidin consists of a N- and C- terminally shortened form, called core streptavidin (Argaraña et al., 1986) comprising the sequence from Ala^{13} or Glu^{14} to Ala^{138} to Ser^{139} of the mature polypeptide. Core streptavidin is more soluble than the full length protein and its binding activity for biotinylated proteins is significantly enhanced (Bayer et al., 1989).

The nucleotide sequence for the intact streptavidin gene from *Streptomyces avidinii* was obtained from Genbank accession no. X03591 (Argaraña et al., 1986).

The structural gene encoding core streptavidin was amplified from chromosomal DNA of *S. avidinii* (ATCC27419) using *Pfu* DNA polymerase and oligonucleotides able to recognise the 5´ and 3´ sequences of the core streptavidin (codons from Ala^{13} to Ser^{139}). The oligonucleotide primers also contained sequences flanking the 5´ and 3´ streptavidin sequences for restriction sites (in particular *Nco*I at the 5´end and *Not*I at the 3´ end to enable the core streptavidin gene to be inserted into the vector pGC (Coia et al., 1996) which already contains the sequence for the human IgM C_H3 domain, in the configuration streptavidin-C domain. The sequence coding for the FLAG® tag epitope (Hopp et al., 1998) lies 3´ to the C domain to enable the FLAG® tag to be expressed as a C-terminal peptide on the molecule.

The amplified core streptavidin gene was inserted into the PCR-Script™ SK(+) plasmid using the PCR-Script™ Cloning Kit obtained from Stratagene, La Jolla. CA (Cat no. 211190-5). After the DNA sequence was confirmed in positive transformants, the core streptavidin sequence was excised from the plasmid by double digestion with *Nco*I and *Not*I, and ligated into a likewise digested pGC vector containing the DNA sequence for human IgM C_H3 domain.

The verified sequence of the expression cassette in pGC comprising the pel B leader sequence, core streptavidin, human IgM C_H3 domain and FLAG® tag is shown in SEQ ID NO: 4 and Figure 10.

Expression in *E. coli* was performed as described in Example 4. Cells from a 500 ml culture were fractionated into periplasmic fraction, cytoplasmic and membrane fraction. The periplasmic fraction was prepared using the protocol of Minsky et al. (1986). The cell pellet remaining after centrifugation to obtain the periplasmic supernatant was resuspended in TE buffer (10mM Tris HCl pH 7.4, 1mM EDTA) sonicated and centrifuged at 20,000xg to obtain the soluble cytoplasmic fraction and the membrane pellet. Western blot analysis of each of the three fractions using the FLAG® tag as a probe indicated that while the expressed product was present in all three fractions, the membrane pellet contained the highest levels.

The membrane fraction was dissolved in 10 ml 6M guanidinium HCl, pH 1.5 (Schmidt and Skerra, 1994), dialysed twice against 200ml 6M guanidinium HCl, pH 1.5, then twice against 2L PBS at 4°C. After centrifugation to remove insoluble aggregate, the supernatant was fractionated on a Superdex 200 (HR 10/30, Pharmacia LKB Biotechnology)

column run in PBS at 0.5ml/min. The elution profile is shown in Figure 11. All three peaks probed with FLAG® indicating the presence of the product. The first peak is high molecular weight aggregate eluting at the void volume of the column. The second and third peaks were collected separately and labelled preparation B (0.10 mg/ml)and preparation A (0.17 mg/ml) respectively. Both preparations showed bands on Western blot of identical size, and so the size difference between the two preparations is related to the multimerisation state of the product. Both preparations were used in tests as described below.

Use of Control Reagent Streptavidin-$C_H3\mu$ in PanBio Dengue Indirect ELISA

In this test, a complex is formed between biotinylated monoclonal mouse IgG to dengue antigens (13C11-B) and streptavidin linked to human IgM C_H3 domain (strep-$C_H3\mu$) to mimic positive human IgM antibody to dengue, and used as a pseudo positive control in commercial IgM capture Dengue ELISA and indirect IgM Dengue ELISA kits where the response is compared to positive and negative controls provided in the kit.

Biotinylation of mouse monoclonal anti dengue IgG (Clone 13C11)

2.56 mg of 13C11 Monoclonal antibody (IgG fraction) to dengue antigen (PanBio Ltd, Windsor, Qld; product 13C7001) was equilibrated in 1ml 50mM sodium bicarbonate buffer, pH 8.0. To this was added 75μl freshly prepared EZ-Link™ Sulfo-NHS-LC-Biotin solution (1mg/ml in water) (Pierce Chemical Company, Rockford, IL; product code 21335), and incubated at room temperature for 1 hour. After the sample had undergone extensive dialysis against PBS (phosphate buffered saline, final sample volume 1.2ml), the protein concentration was estimated by absorbance at 280nm to be 1.6 mg/ml.

The success of the biotinylation reaction was confirmed by ELISA, in which dilutions of the biotinylated 13C11 antibody was added to wells containing immobilised dengue antigen. Non biotinylated 13C11 was used as a negative control. A streptavidin-horse radish peroxidase conjugate was used to visualise the presence of the biotinylated 13C11 antibody.

Dengue IgM Indirect ELISA

The reagents used in this assay were as follows:

- ELISA plate coated with dengue 2 antigen (PanBio Pty Ltd, Windsor Qld)

- IgM Positive Control Serum, IgM Cut-off Calibrator Serum, Negative Control Serum for IgM (PanBio Pty Ltd provided in the kit)
- Biotinylated mouse anti dengue IgG (clone 13C11), concentration = 1.6 mg/ml (biotinylation was performed as described above).
- Non biotinylated mouse anti dengue IgG (clone 13C11), concentration = 1.6 mg/ml
- Control Linker Reagent: Strep-$C_H3\mu$ Preparation A, concentration 0.17mg/ml.

Control linker reagent was mixed with biotinylated and non-biotinylated 13C11 Mab prior to the assay in the following proportions:

1. 13C11-Biotin (1/10,000) + Control Linker Prep A 1/10
2. 13C11-Biotin (1/10,000) + Control Linker Prep A 1/100
3. 13C11-Biotin (1/10,000) + Control Linker Prep A 1/1000
4. 13C11 (1/10,000) [nonbiotinylated] + Control Linker Prep A 1/10
5. 13C11 (1/10,000) [nonbiotinylated] + Control Linker Prep A 1/100
6. 13C11 (1/10,000) [nonbiotinylated] + Control Linker Prep A 1/1000

Dilution was in serum diluent (Tris buffered saline with preservatives and additives) as supplied in the kit. Mixing took place at room temperature for 10 min with rotation.

Additions to ELISA plate were in the following order:

1. Samples as above, blank, positive, negative and cut-off serum controls (100μl) incubated at 37°C for 1 hour, followed by 6 washes with diluted wash buffer.

2. HRP-labelled sheep anti Human IgM (Silenus/AMRAD; Code MAH) 100μl of 1/1000 dilution; incubated for 1 hour at 37°C followed by 6 washes with diluted wash buffer.

3. Reaction was developed with 100 μl TMB reagent 10 min at room temperature, followed by the addition of 100 μl 1M phosphoric acid. Samples were read at 450nm.

The results obtained from this indirect assay are shown in Table 7.

Table 7

Sample	A450
Blank (Serum diluent only)	0.058
Negative serum Control 1/100	0.063
Positive Serum Control 1/100	1.419
Positive Cut-Off 1/100	0.522
Positive Cut-Off 1/100 (duplicate)	0.580
Control Linker 1/10 + 13C11-biotin	1.186
Control Linker 1/10 + 13C11-biotin (dup)	1.109
Control Linker 1/100 + 13C11-biotin	1.070
Control Linker 1/100 + 13C11-biotin (dup)	1.076
Control Linker 1/1000 + 13C11-biotin	0.212
Control Linker 1/1000 + 13C11-biotin (dup)	0.183
Control Linker 1/10 + 13C11	0.151
Control Linker 1/100 + 13C11	0.059
Control Linker 1/1000 + 13C11	0.074

These results show tha the complex formed between the Strep-$C_H3\mu$ linker reagent and the biotinylated mouse anti dengue IgG (13C11) acted as a human positive control up to a dilution of at least 1/100. No reaction was observed if the mouse monoclonal IgG was not biotinylated, or if there was insufficient control linker reagent to capture the biotinylated mouse Mab.

Use of Control Reagent Streptavidin-$C_H3\mu$ in PanBio Dengue IgM Capture ELISA

The Dengue IgM capture ELISA test kit from PanBio Ltd (Windsor, Qld; Cat No DEMF-200) was used for this demonstration. The positive and cut-off control sera provided contain human IgM antibodies to dengue. The negative control serum contains human IgM antibodies, but with no specificity for dengue antigens.

Two test samples of the Streptavidin-Human C_H3 μ domain linker reagent were used:

a) Preparation A, 0.17 mg/ml

b) Preparation B, 0.10 mg/ml

These preparations were mixed with biotinylated and non-biotinylated 13C11 Mab as described below.

The following samples were prepared for ELISA:

1. Blank – 100 μl serum diluent only, provided in PanBio kit
2. Negative Control Serum–from PanBio kit; 100 μl, diluted 1/100 in serum diluent.
3. IgM Positive Control Serum–from PanBio kit; 100 μl, diluted 1/100 in serum diluent.
4. Cut-off Calibrator–from PanBio kit; 100 μl, diluted 1/100 in serum diluent.
5. Strep-$C_H3\mu$ Preparation A + 13C11-Biotin: 10μl of prepA diluted to 1ml with serum diluent to which 1μl 13C11-Biotin (1/1000 dilution) was added (Final concentration of PrepA protein = 1.7 μg/ml; 13C11-Biotin = 1.6 μg/ml).
6. Strep-$C_H3\mu$ Preparation B + 13C11-Biotin: 10μl of prepB diluted to 1ml with serum diluent to which 1μl 13C11-Biotin (1/1000 dilution) was added (Final concentration of PrepB protein = 1.0 μg/ml; 13C11-Biotin = 1.6 μg/ml).
7. Blank + 13C11-Biotin (negative control) 1μl 13C11-Biotin was added to 1ml serum diluent (Final concentration of 13C11 biotin = 1.6 μg/ml).
8. Strep-$C_H3\mu$ Preparation A + 13C11(non biotinylated) (negative control): 10μl of prepA diluted to 1ml with serum diluent to which 1μl 13C11 (1/1000 dilution) was added (Final concentration of PrepA protein = 1.7 μg/ml; 13C11 = 1.6 μg/ml).
9. Strep-$C_H3\mu$ Preparation B + 13C11(non biotinylated) (negative control): 10μl of prepB diluted to 1ml with serum diluent to which 1μl 13C11 (1/1000 dilution) was added (Final concentration of PrepB protein = 1.0 μg/ml; 13C11 = 1.6 μg/ml).
10. Blank + 13C11 (negative control) 1μl 13C11-Biotin was added to 1ml serum diluent (Final concentration of 13C11 biotin = 1.6 μg/ml).

Each sample was mixed on a rotating wheel for 10 min at room temperature, then 100 μl of each (some in duplicate) were added to ELISA strips from the test kit which were pre-coated with polyclonal sheep anti human IgM. The strips were covered and incubated at 37°C for 60 min, then washed three times for 2 min with PBS containing 0.05% Tween 20.

At the same time as the above incubation, 2ml conjugated monoclonal antibody tracer (PanBio: anti dengue–HRP) was added to one vial of

lyophilised dengue antigen (serogroups 1-4) and rocked gently at room temperature to aid in the dissolution of the dengue antigen. After the above washes, 100µl of the HRP conjugate was added to each well, incubated for 60 min at 37°C then washed three times for 2 min with PBS-0.05% Tween 20. 100µl of TMB reagent (3´,3´,5´,5´ tetramethylbenzidine/hydrogen peroxide; supplied with the kit) was then added to each well and the strips incubated at room temperature for 10 mins. The reaction was stopped by the addition of 100µl 1M phosphoric acid and the colour intensity read at 450 nm. Results of this assay are shown in Table 8.

Table 8

Sample	A450
Blank (serum diluent only)	0.115
Negative serum control	0.121
IgM positive serum control	2.678
Positive Cut-off Calibrator	1.198
Positive Cut-off Calibrator (duplicate)	1.235
Preparation A + 13C11-biotin	1.914
Preparation A + 13C11-biotin (duplicate)	1.850
Preparation B + 13C11-biotin	1.200
Preparation B + 13C11-biotin (duplicate)	1.344
Blank + 13C11-biotin	0.115
Blank + 13C11-biotin (duplicate)	0.128
Preparation A + 13C11(non biotinylated)	0.142
Preparation A + 13C11(non biotinylated)	0.126
Preparation B + 13C11(non biotinylated)	0.113
Preparation B + 13C11(non biotinylated)	0.124
Blank + 13C11(non biotinylated)	0.106

Both preparations of Strep-$C_H3\mu$ gave positive reactions in the ELISA at levels sufficient for the complex with mouse IgG to be used as a replacement for the positive control serum. The lower reading with preparation B can be partly attributed to the lower concentration of the product. Results with the controls indicated that there were no significant background problems.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References:

Åkerström, B. and Björck, L (1989) Protein L: an immunoglobulin light chain-binding bacterial protein. Characterization of binding and physicochemical properties. J. Biol. Chem. 264: 19740-19746.

Argaraña, C.E., Kuntz, I.D., Birken, S., Axel, R. and Cantor, C.R. Molecular cloning and nucleotide sequence of the streptavidin gene. Nucl. Acids Res. 14 1871-1882.

Bayer, E.A., Ben-Hur, H., Hiller, Y., and Wilchek, M. (1989) Post-secretory modifications of streptavidin. Biochem. J. 259 369-376.

Better, M., Chang, C.P., Robinson, R.R., and Horwitz, A.H. (1988) Escherichia coli secretion of an active chimeric antibody fragment. Science 240, 1041-1043.

Björck, L. and Kronvall, G. (1984) Purification and some proerties of streptococcal protein G, a novel IgG-binding reagent. J. Immunol. 133, 969-974.

Borza, D-B., Tatum, F.M. and Morgan, W.T. (1996) Domain Structure and Conformation of Histidine-Proline-Rich Glycoprotein. Biochemistry 35 1925-1934.

Boulianne, G.L., Hozumi, N. and Shulman, M.J. (1984) Production of functional chimaeric mouse/human antibody. Nature (Lond.) 312, 643-646.

Boyle, M.D.P. and Reis, K.J. (1987) Bacterial Fc receptors. Bio/technology 5, 697-703.

Coia G., Hudson, P. J. and Lilley G. G. (1996) Construction of recombinant extended single-chain antibody peptide conjugates for use in the diagnosis of HIV1 and HIV2. J. Immunol. Methods. 192, 13-23.

Diesenhofer, J., (1981). Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from Staphylococcus aureus at 2.9- and 2.8 Å resolution. Biochemistry 20, 2361-2370.

Dolezal, O., Coia, G., Guthrie, R.E., Lilley, G.G. and Hudson, P.J. (1995) Escherichia coli expression of a bifunctional Fab-peptide epitope reagent for the rapid diagnosis of HIV-1 and HIV-2 Immunotechnology 1 197-209.

Dorai, H. and Gillies, S.D. (1989) The complete nucleotide sequence of a human immunoglogulin genomic C μ gene. Nucleic Acids Research 17, 6412

Gorgani, N.N., Parish, C.R., Easterbrook Smith, S.B., and Altin, J.G. (1997) Histidine-Rich Glycoprotein binds to Human IgG and C1q and inhibits the formation of insoluble immune complexes. Biochemistry 36 6653-6662.

Green, N.M. (1990) Avidin and Streptavidin. Methods in Enzymol. 184 51-67.

Hamilton. R.G. (1990) Engineered human antibodies as immunologic quality control reagents. Ann. Biol. Clin. 48, 473-477.

Hamilton R.G. (1991) Application of engineered chimeric antibodies to the calibration of human antibody standards. Ann. Biol. Clin. 49, 242-248.

Heusser C.H., Anderson, C.L. and Grey, H.M. (1977) Receptors for IgG: subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a macrophage cell line. J. Exp. Med 145, 1316-1327.

Hogarth, P.M., Hibbs, M.L., Bonadonna, L., Scott, B.M., Witort, E. Pieterz, G.A. and McKenzie, I.F. (1987) Immunogenetics 26, 161-168.

Hopp, T. P., Prickett, K. S., Price, V.L., Libby, R. T., March C. J., Cerretti, D. P., Urdal, D. L. and Conlon, P. J. (1988). A short polypeptide marker sequence useful for recombinant protein identification and purification. Bio/Technology 6, 1204-1210.

Huston, J. S., Mudgett-Hunter, M., Tai,M.-S., McCartney, J. E., Warren, F. D., Haber, E., and Oppermann, H (1991) Protein engineering of single chain Fv analogs and fusion proteins. Methods Enzymol. 203, 46-88.

Kulcczycki, A. Jr., Webber, J., Soares, H.A., Onken, M.D., Thompson, J.A., Chaplin, D.D., Loh, D.Y. and Tillinghast J.P. (1990) Genomic organization of mouse Fc receptor genes. Proc. Natl. Acad. Sci. USA 87, 2856-2960.

Minsky, A., Summers, R.G. and Knowles, J.R. (1986) Secretion of beta lactamase into the periplasm of Escherichia coli: Evidence for a distinct release step associted with a conformational change. Proc. Nat.l Acad. Sci. USA 83 4180-4184.

Morrison, S.L., Johnson, M.J. Herzenberg, L.A. and Oi, V.T. (1984) Chimeric human antibodies: mouse antigen binding domains with human constant regions. Proc Natl. Acad. Sci. USA 81, 6851-6855.

Pähler, A., Hendrickson, W.A., Kolks, M.A., Aragaña, C.E., and Cantor C.R. Characterization and crystallization of core streptavidin J. Biol. Chem. 262 13933-13937.

Reichmann, L., Clark, M., Waldmann, H. and Winter G. (1998) Reshaping human antibodies for therapy. Nature (Lond.) 332, 323-327.

Reis, K.J., Ayoub, E.M., and Boyle, M.D.P. (1984) Streptococcal Fc Receptors. II Comparison of the reactivity of a receptor from a group C Streptococcus with staphylococcal protein A. J. Immunol. 132, 3098-3102.

Rylatt, D. B., Kemp, B. E., Bundesen, P. G., John, M. A., O'Reilly, E. J., Cottis, L. E., Miles, S. J., Khan, J. M., Dinh, D. P., Stapleton, D. and Hillyard, C. J. (1990). A rapid whole-blood immunoassay system. Med. J. Aust. 152, 75-77.

Sang, C.T., Cuzzubbo, A.J. and Devine, P.L. (1998) Evaluation of commercial capture Enzyme -Linked Immunosorbent Assay for the determination of IgM

and IgG antibodies produced during dengue infection. Clinical Diagnostic Laboratory Immunology 5, (1) in press.

Schmidt, T.G.M. and Skerra, A. (1994) One-step affinity purification of bacterially produced proteins by means of the “Strep tag” and immobilized recombinant core streptavidin. J Chromatog. A 676, 337-345.

Segal D.M., and Titus, J.A. (1978) The subclass specificity for the binding of murine myeloma proteins to macrophages and lymphocyte cell lines and to normal spleen cells J. Immunol. 120, 1395-1403.

Skerra A. (1993) Bacterial expression of immunoglobulin fragments, Curr. Opin. Immunol. 5, 256-262.

Uhlen, M., Guss, B., Nilsson, B., Gatenvack, S., Philipson, L and Lindberg, M. (1984) Complete sequence of the Staphylococcal gene encoding Protein A. J. Biol. Chem. 259, 1695-1702.

Unkeless, J.C., Scigliano, E. and Freedman, V.H. (1988) Structure and function of human and murine receptors for IgG (1988) Ann. Rev. Immunol 6, 251-281.

Winter, G. and Milstein, C. (1991) Man-made antibodies. Nature (Lond.) 349, 293-299.

Yanisch-Perron, C., Vieira, J., and Messing, J.(1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33, 103-119.

Zhou, H., Fisher, R.J. and Papas, T.S. (1994) Optimization of primer sequences for mouse repertoire display library construction. Nucleic Acids Research 22, 888-889.

Claims:

1. A chimeric antibody conjugate comprising an antigen binding region of a non-human antibody and an immunoglobulin constant region which comprises at least one C_H domain or epitope thereof, with the proviso that the constant region is not a naturally occurring F_C fragment.

2. A chimeric antibody conjugate according to claim 1 in which the non-human antigen binding region comprises or consists of a non-human Fab fragment or part thereof.

3. A chimeric antibody conjugate according to claim 1 or claim 2 in which the non-human antigen binding region comprises or consist of an scFv fragment.

4. A chimeric antibody conjugate according to any one of claims 1 to 3 in which the non-human antigen binding region is derived from a mouse.

5. A chimeric antibody conjugate according to any one of claims 1 to 4 in which the constant region is derived from a human antibody.

6. A chimeric antibody conjugate according to any one of claims 1 to 5 in which the constant region comprises one or more constant domains derived from an IgM antibody.

7. A chimeric antibody conjugate according to claim 6 in which the constant region comprises one or more $C_H3\mu$ domains.

8. A chimeric antibody conjugate according to any one of claims 1 to 5 in which the constant region comprises one or more constant domains derived from an IgG antibody.

9. A chimeric antibody conjugate according to claim 8 in which the constant region comprises one or more $C_H3\gamma$ domains.

10. A chimeric antibody conjugate according to any one of claims 1 to 5 in which the constant region comprises one or more constant domains derived from an IgA antibody.

11. A chimeric antibody conjugate according to any one of claims 1 to 10 in which the constant region comprises a non-naturally occurring combination of C_H domains or epitopes thereof.

12. A chimeric antibody conjugate according to any one of claims 1 to 11 in which the non-human antigen binding region binds to an epitope of an infectious agent selected from dengue virus, rubella virus, herpes virus, parvovirus, human glycophorin, *Rickettsia sibirica, Burkholeria pseudomallei, Salmonella typhi* or *paratyphi, Leptospira interrogans, Plasmodium falciparum/vivax,* Japanese encephalitis virus, Yellow fever virus, *Bordetella pertussis/parapertussis, Candida albicans/kruzei,* Varicella zoster virus, HIV, Hepatitis viruses, Human papilloma virus, Epstein-Barr virus, Ross River virus, *Brucella abortis,* Human herpesvirus-6, Parvovirus B19, *Coxiella burnettii,* Herpes simplex viruses 1&2, *Rickettsia rickettsii, Conori australis,* and *Rickettsia tsutsugamushi.*

13. A recombinant polynucleotide molecule comprising a sequence encoding a non-human V_H region, a sequence encoding a non-human V_L region, a sequence encoding a flexible linker positioned between the V_H region sequence and the V_L region sequence, and a heterologous sequence encoding a C_H domain or epitope thereof.

14. A recombinant polynucleotide molecule according to claim 13 in which the heterologous sequence encodes a human C_H domain.

15. A recombinant polynucleotide molecule according to claim 13 or claim 14 in which the C_H domain sequence is linked to the 3' end of the V_L or V_H sequence.

16. A recombinant polynucleotide molecule according to any one of claims 13 to 15 in which the polynucleotide molecule includes a control

sequence which directs the synthesis of both the V_L and V_H polypeptide regions.

17. A recombinant polynucleotide molecule according to claim 16 in which the control sequence is the lac promoter.

18. A recombinant polynucleotide molecule according to any one of claims 13 to 17 in which the molecule includes a sequence encoding a leader peptide which directs the synthesised polypeptide chains to the host cell periplasm.

19. A recombinant polynucleotide molecule according to claim 18 in which the leader sequence is the pel B sequence.

20. A recombinant polynucleotide molecule comprising a sequence encoding a non-human V_L region, a sequence encoding a non-human C_L region, a sequence encoding a non-human V_H region, a heterologous sequence encoding a C_H domain or epitope thereof, and optionally a sequence encoding a non-human C_H1 region.

21. A recombinant polynucleotide molecule according to claim 21 in which the heterologous sequence encodes a human C_H domain.

22. A recombinant polynucleotide molecule according to claim 20 or claim 21 in which the V_L and C_L sequences are linked together so that the V_L and C_L regions are expressed as a single polypeptide.

23. A recombinant polynucleotide molecule according to any one of claims 20 to 22 in which the V_H and C_H1 sequences are linked together so that the V_H and C_H1 regions are expressed as a single polypeptide.

24. A recombinant polynucleotide molecule according to any one of claims 20 to 23 in which the polynucleotide molecule includes a control sequence which directs the synthesis of both the V_L-C_L and V_H-C_H1 polypeptide regions.

25. A recombinant polynucleotide molecule according to claim 24 in which the control sequence is the lac promoter.

26. A recombinant polynucleotide molecule according to any one of claims 20 to 25 in which the polynucleotide molecule includes a sequence encoding a leader peptide which directs the synthesised polypeptide chains to the host cell periplasm.

27. A recombinant polynucleotide molecule according to claim 26 in which the leader sequence is the pel B sequence.

28. A recombinant polynucleotide molecule according to any one of claims 20 to 27 in which the heterologous C_H domain sequence is linked to the V_L-C_L sequences or the V_H-C_H1 sequences so that the expressed heterologous C_H domain is attached to the V_L-C_L polypeptide or the V_H-C_H1 polypeptide.

29. A vector comprising a poynucleotide according to any one of claims 13 to 29.

30. A bacterial, yeast, insect or mammalian host cell transformed with a vector according to claim 29.

31. A method of producing a chimeric antibody conjugate which comprises culturing a host cell according to claim 30 under conditions enabling the expression of the conjugate and optionally recovering the conjugate.

32. A chimeric antibody conjugate produced by a method according to claim 31.

33. A method for detecting an antibody in a biological sample which involves comparing the level of detection obtained with the biological sample to the level of detection obtained with a positive control, wherein the positive control comprises a chimeric antibody conjugate according to any one of claims 1 to 12.

34. A method according to claim 33 in which the biological sample is a human biological sample.

35. A method according to claim 33 or claim 34 in which the antibodies to be detected are antibodies characteristic of a disease selected from dengue fever, japanese encephalitis, rubella, spotted fever, herpes infection, parvovirus infections, melioidosis, typhoid, leptospirosis, malaria, yellow fever, whooping cough, systemic candidiasis/thrush, chicken pox, shingles, AIDS, hepatitis, liver cancer, cervical cancer, infectious mononucleosis, nasopharyngeal carcinoma, Ross River fever, brucella, exanthum subitum (sixth disease/roseola infantum), erythema ingfectiosum (fifth disease), Q fever, cold sores, genital herpes, spotted fever and scrub typhus.

36. A method according to any one of claims 33 to 35 in which the antibody is an IgM antibody.

37. A method according to any one of claims 33 to 35 in which the antibody is an IgG antibody.

38. A method according to any one of claims 33 to 35 in which the antibody is an IgA antibody.

39. A bifunctional molecule for use in labelling an antibody derived from a first species, the bifunctional molecule comprising a binding region which binds to the antibody of the first species or to one or more groups provided thereon, and a constant region derived from an antibody of a second species, the constant region comprising at least one C_H domain or an epitope thereof.

40. A bifunctional molecule according to claim 39 in which the binding and constant regions are separated by a linker molecule.

41. A bifunctional molecule according to claim 40 in which the linker molecule is a peptide of between 1 and 20 amino acids in length.

42. A bifunctional molecule according to claim 41 in which the linker molecule is a peptide of between 2 and 5 amino acids in length.

43. A bifunctional molecule according to any one of claims 39 to 42 in which the binding region is not derived from an antibody.

44. A bifunctional molecule according to any one of claims 39 to 43 in which the binding region binds directly to the antibody derived from the first species.

45. A bifunctional molecule according to claim 44 in which the binding region is derived from a protein selected from the group consisting of, *Streptococcal* protein G, *Staphylococcal aureus* protein A and *Peptostreptococcus magnus* protein L.

46. A bifunctional molecule according to claim 45 in which the binding region comprises fragment B of *Staphylococcus aureus* protein A.

47. A bifunctional molecule according to claim 44 in which the binding region comprises a mouse Fc γ receptor or fragment thereof.

48. A bifunctional molecule according to claim 44 in which the binding region comprises a histidine rich glycoprotein.

49. A bifunctional molecule according to any one of claims 39 to 43 in which the binding region binds to one or more groups provided on the antibody of the first species.

50. A bifunctional molecule according to claim 49 in which the group(s) is a biotin molecule and the binding region comprises streptavidin or a fragment thereof.

51. A bifunctional molecule according to any one of claims 39 to 50 in which the antibody constant region is not a naturally occurring Fc fragment.

52. A bifunctional molecule according to any one of claims 39 to 51 in which the constant region comprises one or more constant domains derived from an IgM antibody.

53. A bifunctional molecule according to claim 52 in which the constant region comprises one or more $C_H3\mu$ domains.

54. A bifunctional molecule according to any one of claims 39 to 51 in which the constant region comprises one or more constant domains derived from an IgG antibody.

55. A bifunctional molecule according to claim 54 in which the constant region comprises one or more $C_H3\gamma$ domains.

56. A bifunctional molecule according to any one of claims 39 to 51 in which the constant region comprises one or more constant domains derived from an IgA antibody.

57. A bifunctional molecule according to any one of claims 39 to 56 in which the antibody constant region comprises or consists of a non-naturally occurring combination of immunoglobulin C_H domains or epitopes thereof.

58. A bifunctional molecule according to any one of claims 39 to 56 in which the antibody constant region comprises or consists of a single C_H domain.

59. A bifunctional molecule according to any one of claims 39 to 58 in which the second species is a human.

60. An isolated polynucleotide encoding a bifunctional molecule according to any one of claims 39 to 59.

61. A vector comprising a polynucleotide according to claim 60.

62. A bacterial, yeast, insect or mammalian host cell transformed with a vector according to claim 61.

63. A method of producing a bifunctional molecule which comprises culturing a host cell according to claim 62 under conditions enabling the expression of the bifunctional molecule and optionally recovering the bifunctional molecule.

64. A bifunctional molecule produced by a method according to claim 63.

65. A complex formed between (i) an antibody or biologically active fragment thereof derived from a first species and (ii) a bifunctional molecule, the bifunctional molecule comprising a binding region which binds to the antibody of the first species or to one or more groups provided thereon, and a constant region derived from an antibody of a second species, the constant region comprising at least one C_H domain or an epitope thereof.

66. A complex according to claim 65 in which the binding region has a K_D for the antibody of the first species, or a group provided thereon, of less than 10^{-6} M.

67. A complex according to claim 66 in which the binding region has a K_D for the antibody of the first species, or a group provided thereon, of less than 10^{-8}M.

68. A complex according to any one of claims 65 to 67 in which the bifunctional molecule binds directly to the antibody derived from the first species.

69. A complex according to claim 68 in which the binding region is derived from a protein selected from the group consisting of, *Streptococcal* protein G, *Staphylococcal aureus* protein A and *Peptostreptococcus magnus* protein L.

70. A complex according to claim 69 in which the binding region comprises fragment B of *Staphylococcus aureus* protein A.

71. A complex according to claim 68 in which the binding region comprises a mouse Fc γ receptor or fragment thereof.

72. A complex according to claim 68 in which the binding region comprises a histidine rich glycoprotein.

73. A complex according to any one of claims 65 to 67 in which the binding region binds to one or more groups provided on the antibody of the first species.

74. A complex according to claim 73 in which the group(s) is a biotin molecule and the binding region comprises streptavidin or a fragment thereof.

75. A complex according to any one of claims 65 to 74 in which the constant region comprises one or more constant domains derived from an IgM antibody.

76. A complex according to claim 75 in which the constant region comprises one or more $C_H3\mu$ domains.

77. A complex according to any one of claims 65 to 74 in which the constant region comprises one or more constant domains derived from an IgG antibody.

78. A complex according to claim 77 in which the constant region comprises one or more $C_H3\gamma$ domains.

79. A complex according to any one of claims 65 to 74 in which the constant region comprises one or more constant domains derived from an IgA antibody.

80. A complex according to any one of claims 65 to 79 in which the antibody constant region comprises or consists of a non-naturally occurring combination of immunoglobulin C_H domains or epitopes thereof.

81. A complex according to any one of claims 65 to 79 in which the antibody constant region comprises or consists of a single C_H domain.

82. A complex according to any one of claims 65 to 81 in which the first species is a rat or mouse.

83. A complex according to any one of claims 65 to 82 in which the second species is a human.

84. A method for detecting an antibody in a biological sample which involves comparing the level of detection obtained with the biological sample to the level of detection obtained with a positive control, wherein the positive control comprises a complex according to any one of claims 65 to 83.

85. A method according to claim 84 in which the biological sample is a human biological sample.

86. A method according to claim 84 or claim 85 in which the antibodies to be detected are antibodies characteristic of a disease selected from dengue fever, Japanese encephalitis, rubella, spotted fever, herpes infection, parvovirus infections, melioidosis, typhoid, leptospirosis, malaria, yellow fever, whooping cough, systemic candidiasis/thrush, chicken pox, shingles, AIDS, hepatitis, liver cancer, cervical cancer, infectious mononucleosis, nasopharyngeal carcinoma, Ross River fever, brucella, exanthum subitum (sixth disease/roseola infantum), erythema infectiosum (fifth disease), Q fever, cold sores, genital herpes, spotted fever and scrub typhus.

87. A method according to any one of claims 84 to 86 in which the antibody is an IgM antibody.

88. A method according to any one of claims 84 to 86 in which the antibody is an IgG antibody.

89. A method according to any one of claims 84 to 86 in which the antibody is an IgA antibody.

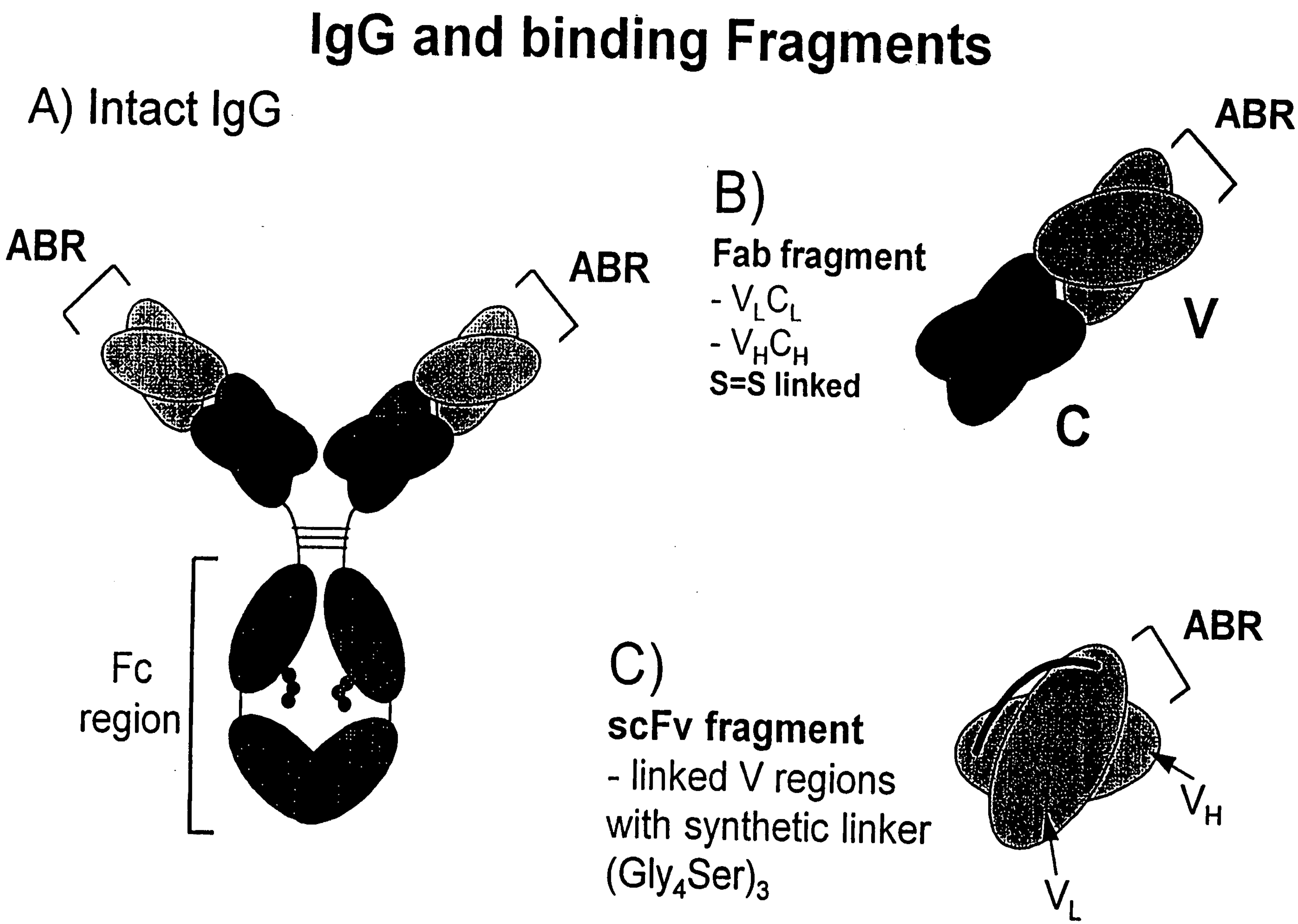

IgG and binding Fragments
A) Intact IgG
ABR
ABR
Fc
region
B)
Fab fragment
- V_LC_L
- V_HC_H
S=S linked
ABR
V
C
C)
scFv fragment
- linked V regions
with synthetic linker
$(Gly_4Ser)_3$
ABR
V_H
V_L

Figure 1

Figure 2

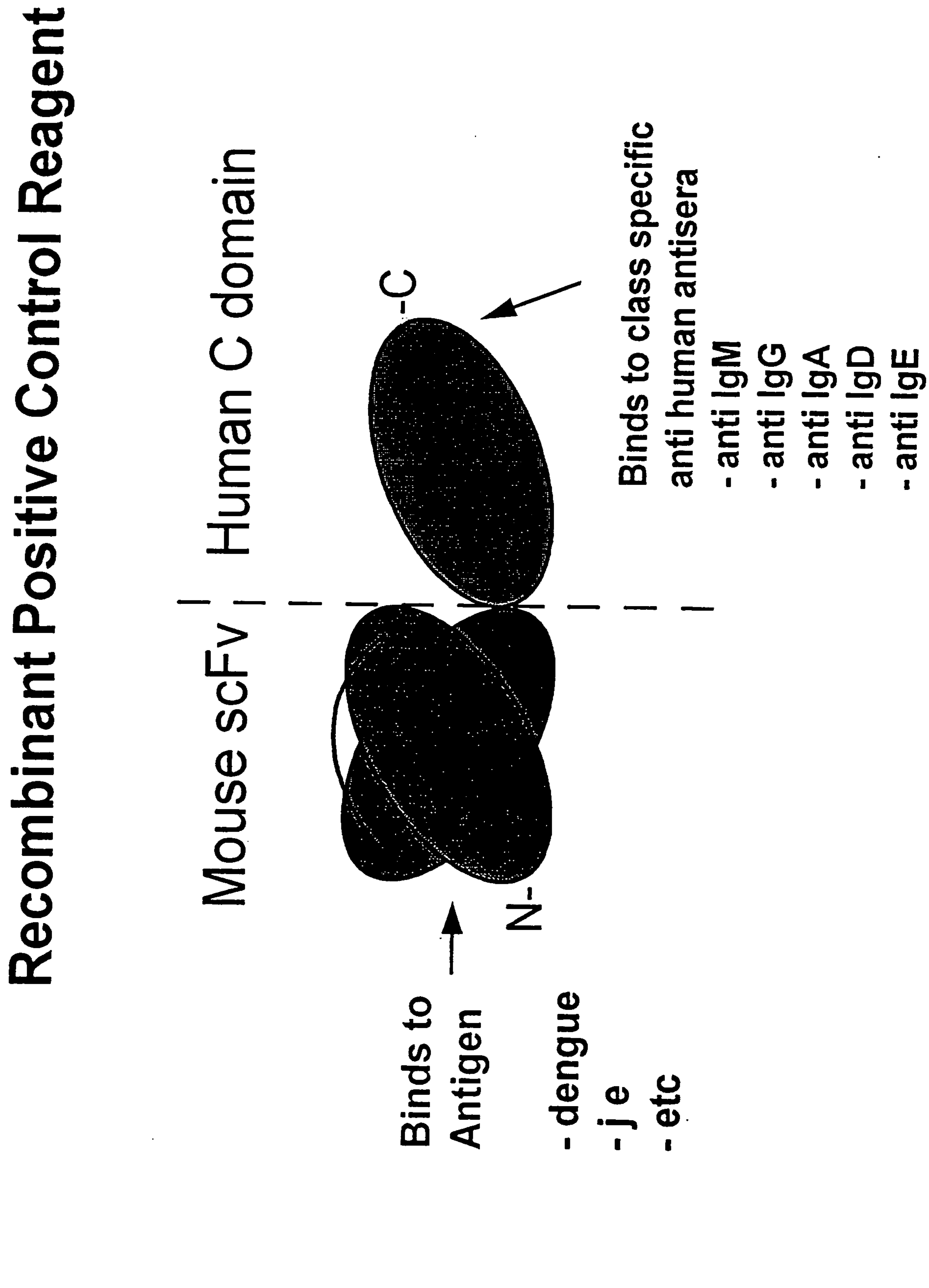
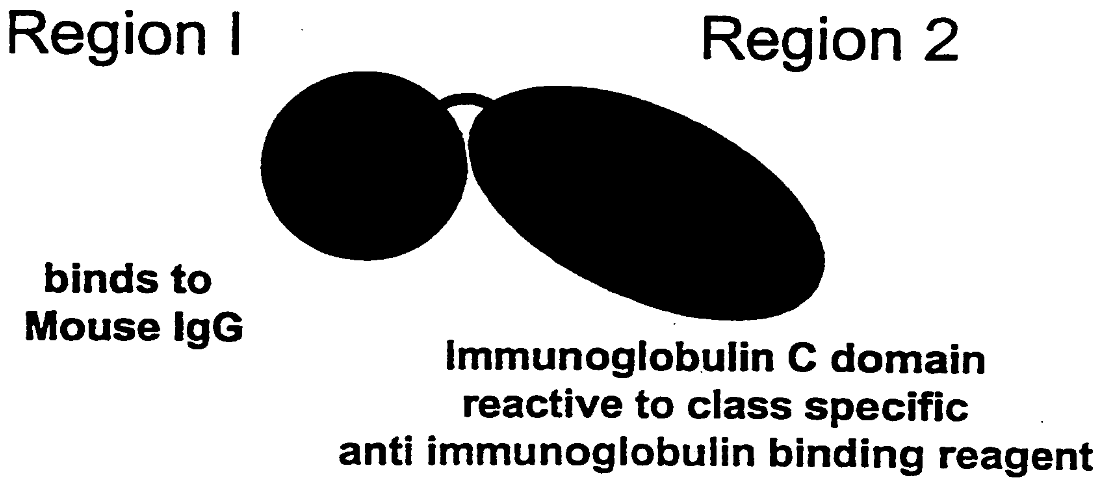

Recombinant Positive Control Reagent
Mouse scFv
Human C domain
-C
N-
Binds to
Antigen
- dengue
- j e
- etc
Binds to class specific
anti human antisera
- anti IgM
- anti IgG
- anti IgA
- anti IgD
- anti IgE

Figure 3



Complex formed between bifunctional molecule and mouse IgG

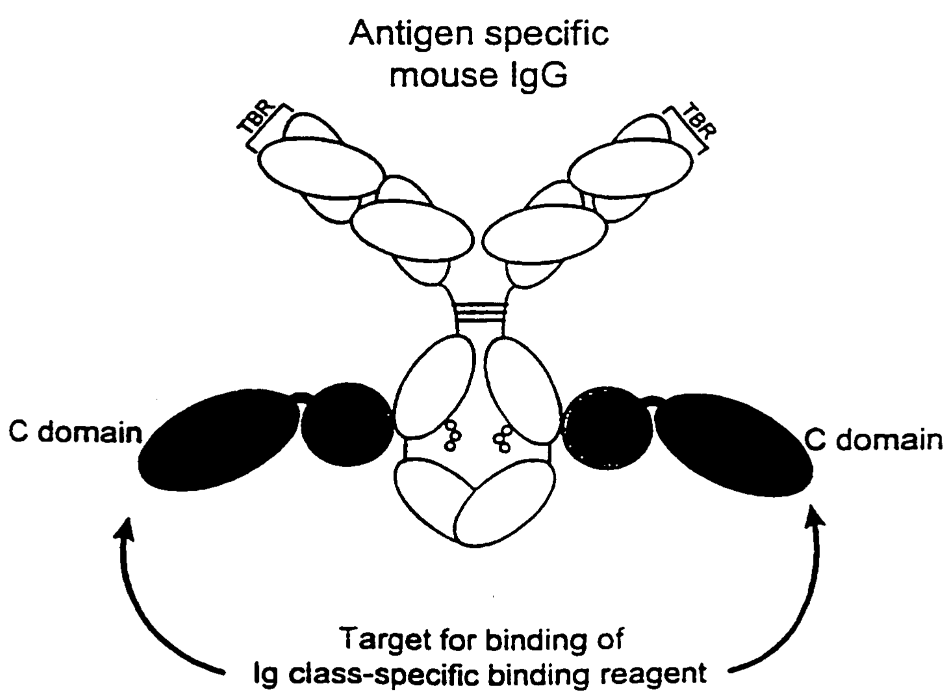


Figure 4

Complex formed between bifunctional molecule containing streptavidin and biotinylated mouse IgG

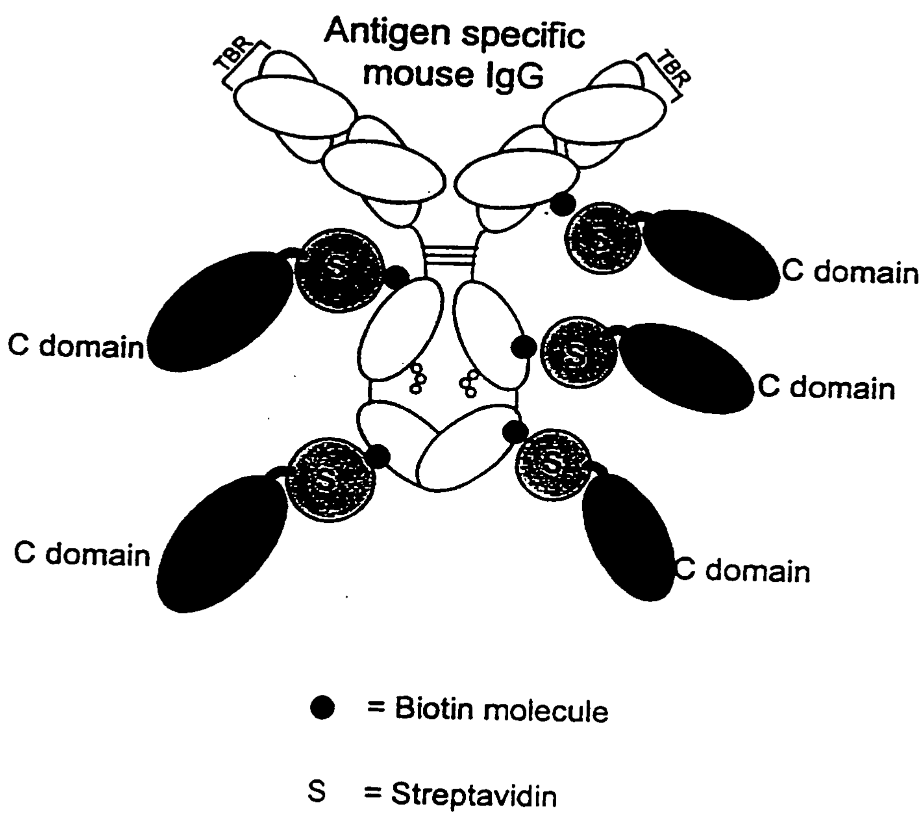


Figure 5

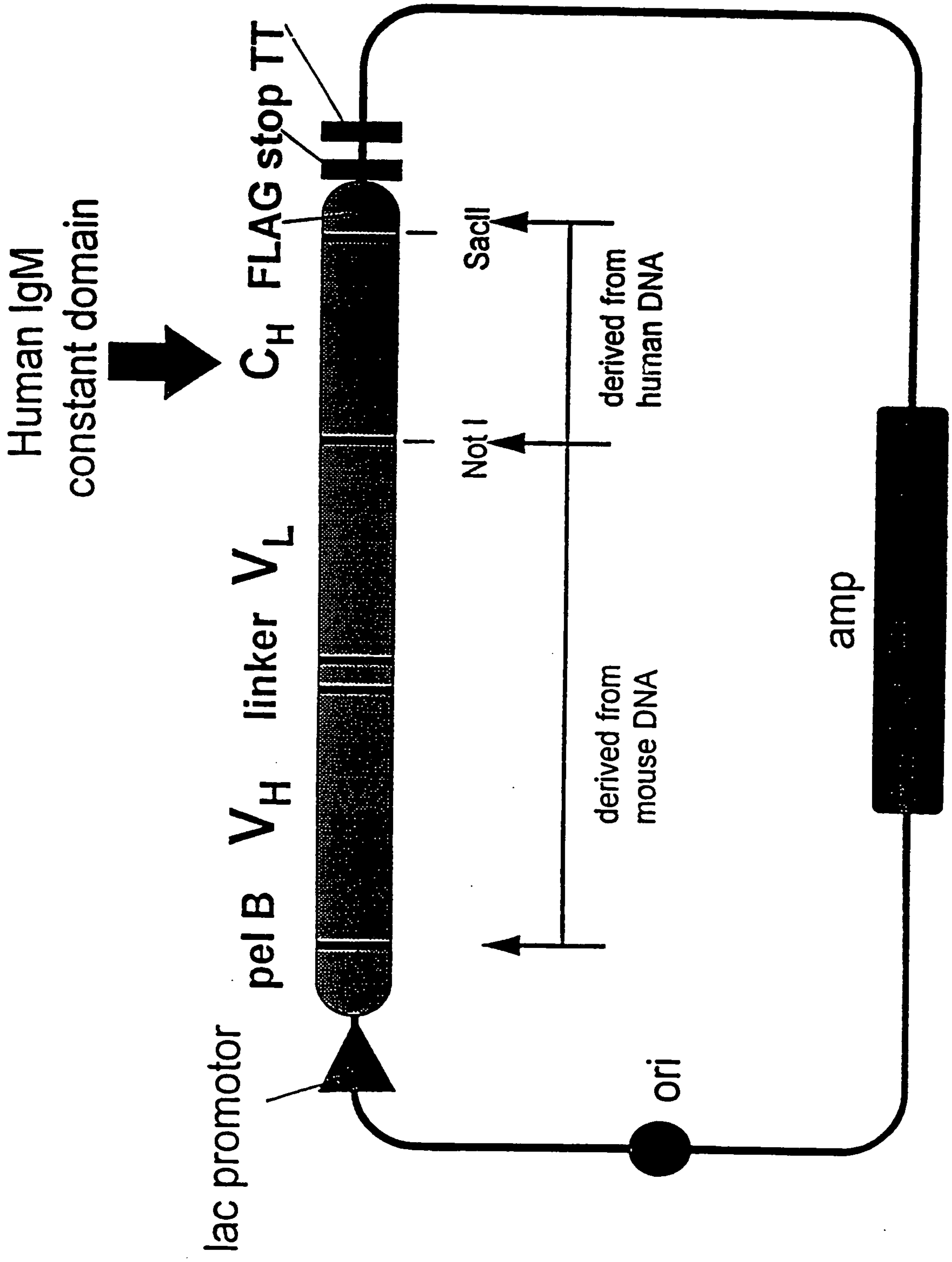

Human IgM
constant domain
lac promotor
pel B
V_H
linker
V_L
C_H
FLAG
stop
TT
SacII
Not I
derived from
human DNA
derived from
mouse DNA
amp
ori

Figure 6

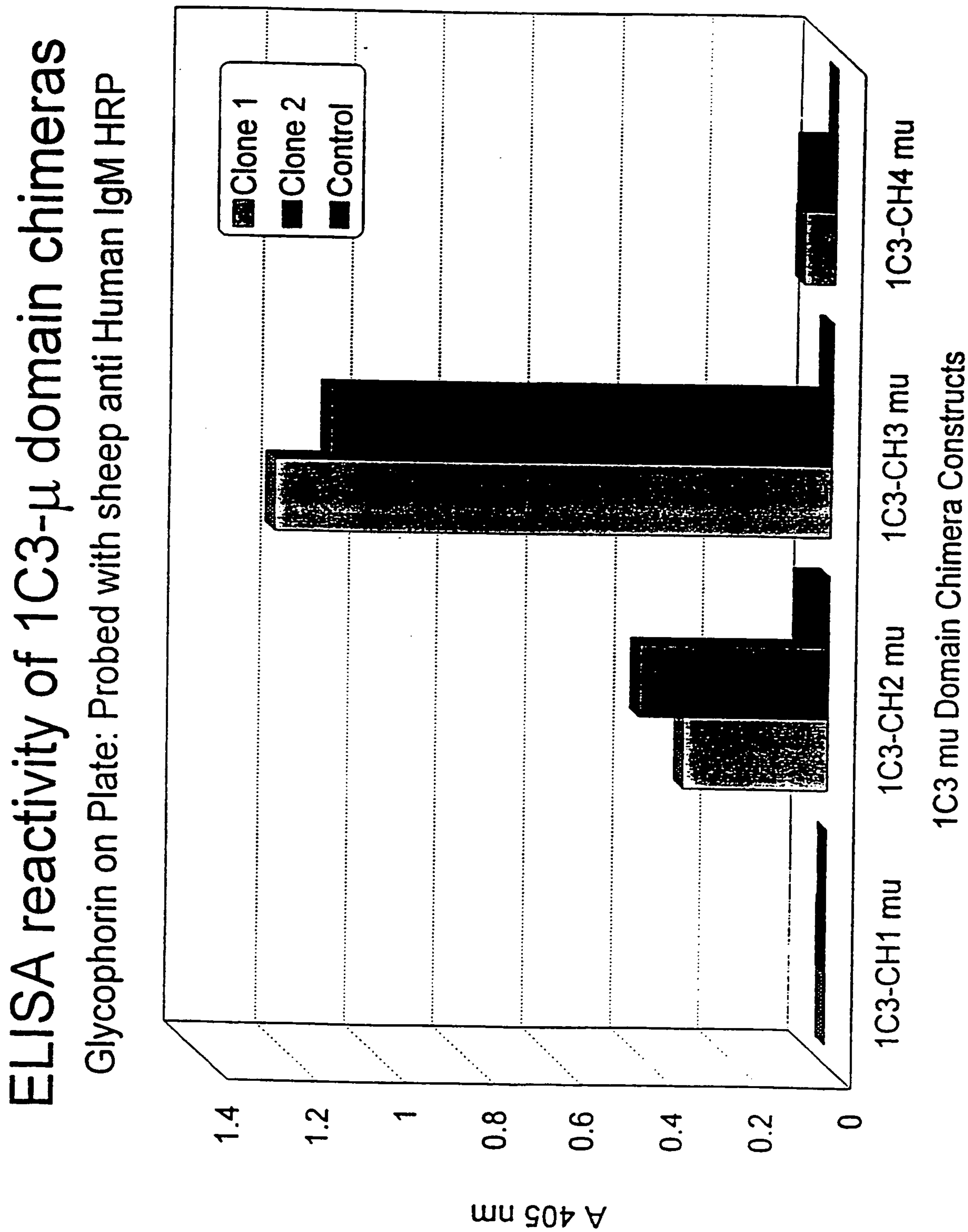

ELISA reactivity of 1C3-μ domain chimeras
Glycophorin on Plate: Probed with sheep anti Human IgM HRP
Clone 1
Clone 2
Control
A 405 nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1C3-CH1 mu
1C3-CH2 mu
1C3-CH3 mu
1C3-CH4 mu
1C3 mu Domain Chimera Constructs

Figure 7

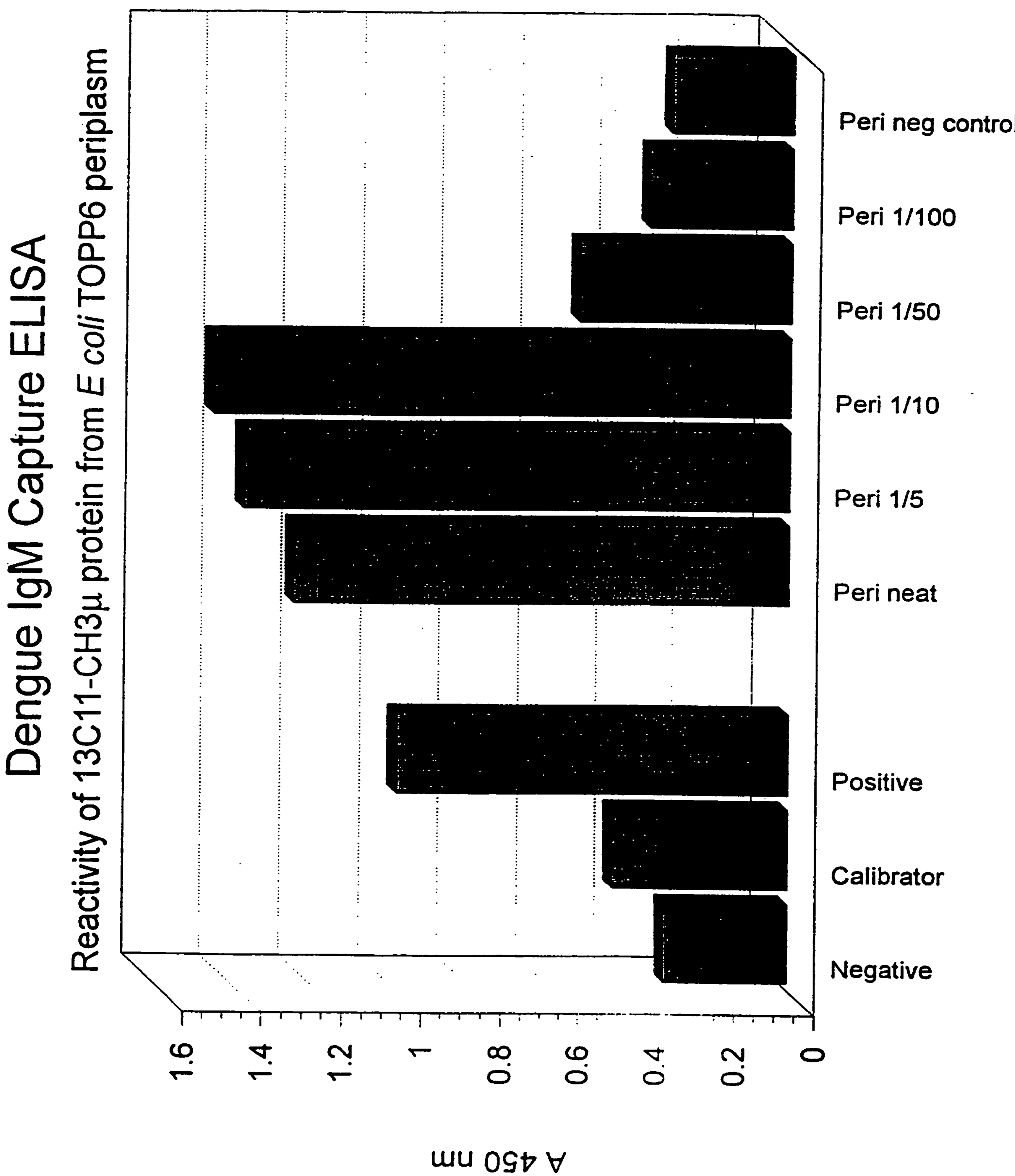

Dengue IgM Capture ELISA
Reactivity of 13C11-CH3μ protein from E coli TOPP6 periplasm
Peri neg control
Peri 1/100
Peri 1/50
Peri 1/10
Peri 1/5
Peri neat
Positive
Calibrator
Negative
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
A 450 nm

Figure 8

```
      M   K   Y   L   L   P   T   A   A   A   G   L   L   L   A
2353 ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG
     \-- Pel B-- -->

      A   Q   P   A   M   A   A   D   N   K   F   N   K   E   Q   Q
2401 GCC CAG CCG GCC ATG GCC GCG GAT AAC AAA TTC AAC AAA GAA CAA CAA
     --- Sfi 1     <N col >   < Start Fragment B

      N   A   F   Y   E   I   L   H   L   P   N   L   N   E   E   Q
2449 AAT GCT TTC TAT GAA ATC TTA CAT TTA CCT AAC TTA AAC GAA GAA CAA

      R   N   G   F   I   Q   S   L   K   D   D   P   S   Q   S   A
2497 CGC AAT GGT TTC ATC CAA AGC CTA AAA GAT GAC CCA AGC CAA AGC GCT

      N   L   L   A   E   A   K   K   L   N   D   A   Q   A   P   K
2545 AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GAT GCT CAA GCA CCA AAA
                                                     End Fragment B->

      S   D   P   A   A   A   D   Q   D   T   A   I   R   V   F   A
2593 AGT GAT CCC GCG GCC GCA GAT CAA GAC ACA GCC ATC CGG GTC TTC GCC
      < linker >    Notl   >  CH3 mu domain

      I   P   P   S   F   A   S   I   F   L   T   K   S   T   K   L
2641 ATC CCC CCA TCC TTT GCC AGC ATC TTC CTC ACC AAG TCC ACC AAG TTG

      T   C   L   V   T   D   L   T   T   Y   D   S   V   T   I   S
2689 ACC TGC CTG GTC ACA GAC CTG ACC ACC TAT GAC AGC GTG ACC ATC TCC

      W   T   R   Q   N   G   E   A   V   K   T   H   T   N   I   S
2737 TGG ACC CGC CAG AAT GGC GAA GCT GTG AAA ACC CAC ACC AAC ATC TCC

      E   S   H   P   N   A   T   F   S   A   V   G   E   A   S   I
2785 GAG AGC CAC CCC AAT GCC ACT TTC AGC GCC GTG GGT GAG GCC AGC ATC

      C   E   D   D   W   N   S   G   E   R   F   T   C   T   V   T
2833 TGC GAG GAT GAC TGG AAC TCC GGG GAG AGG TTC ACG TGC ACC GTG ACC

      H   T   D   L   P   S   P   L   K   Q   T   I   S   R   P   K
2881 CAC ACA GAC CTG CCC TCG CCA CTG AAG CAG ACC ATC TCC CGG CCC AAG

      G   A   A   D   Y   K   D   D   D   D   K   *
2929 GGc GCC GCG GAT TAT AAA GAT GAT GAT GAT AAA TAA GAA TTC AGC CCG
         Sac 2   ------------ FLAG -------------      Eco R1  <-------

2977 CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT TTT ACA ACG
     -------- TrpA terminator ----->
```

Figure 9

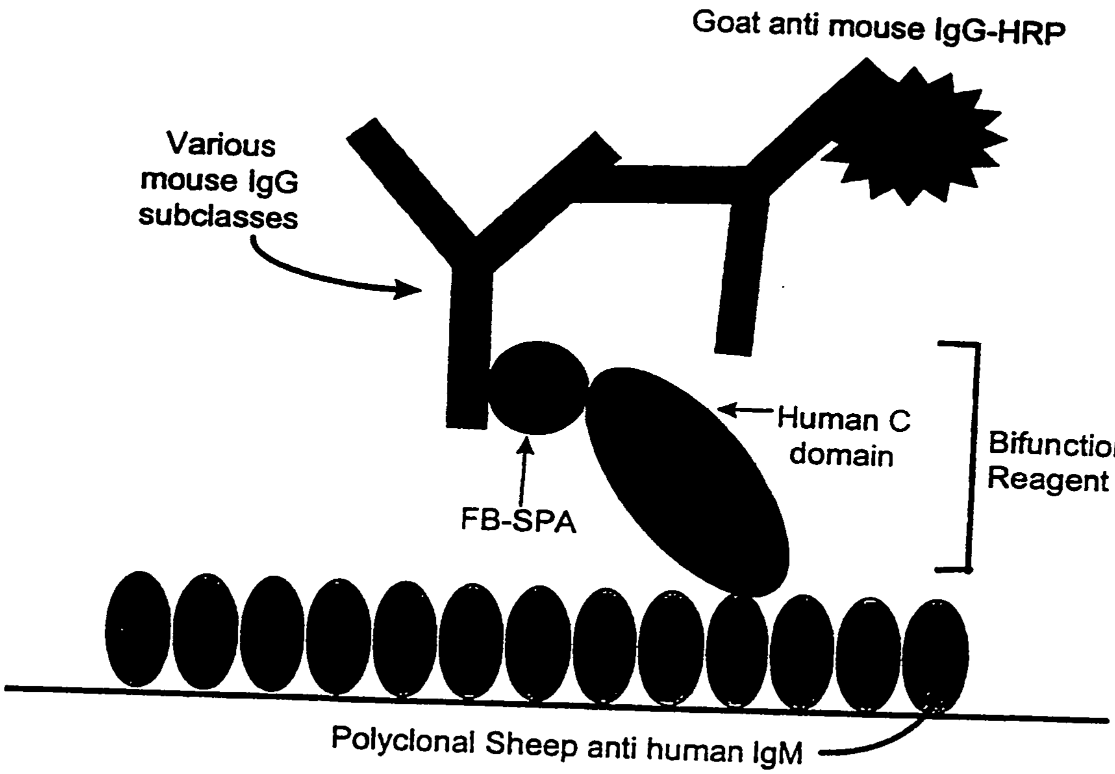

Goat anti mouse IgG-HRP
Various mouse IgG subclasses
Human C domain
Bifunctional Reagent
FB-SPA
Polyclonal Sheep anti human IgM

Figure 10

Sequence of expression cassette Str-$C_H3\mu$ in pGC vector

M K Y L L P T A A A G L L L L A
ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCG
|-- Pel B-- -->

A Q P A M A E A G I T G T W Y N
GCC CAG CCG GCC ATG GCC gag gcc ggc atc acc ggc acc tgg tac aac
--- Sfi I <Nco I >-----core streptavidin --------------->

Q L G S T F I V T A G A D G A L
cag ctc ggc tcg acc ttc atc gtg acc gcg ggc gcc gac ggc gcc ctg

T G T Y E S A V G N A E S R Y V
acc gga acc tac gag tcg gcc gtc ggc aac gcc gag agc cgc tac gtc

L T G R Y D S A P A T D G S G T
ctg acc ggt cgt tac gac agc gcc ccg gcc acc gac ggc agc ggc acc

A L G W T V A W K N N Y R N A H
gcc ctc ggt tgg acg gtg gcc tgg aag aat aac tac cgc aac gcc cac

S A T T W S G Q Y V G G A E A R
tcc gcg acc acg tgg agc ggc cag tac gtc ggc ggc gcc gag gcg agg

I N T Q W L L T S G T T E A N A
atc aac acc cag tgg ctg ctg acc tcc ggc acc acc gag gcc aac gcc

W K S T L V G H D T F T K V K P
tgg aag tcc acg ctg gtc ggc cac gac acc ttc acc aag gtg aag ccg
-end core

S A A S D P A A A D Q D T A I R
tcc gcc gct agc gat ccc gcg gcc gca gat caa gac aca gcc atc cgg
strep-| -----< linker > <-Not I > |----$C_H3\mu$ domain

V F A I P P S F A S I F L T K S
gtc ttc gcc atc ccc cca tcc ttt gcc agc atc ttc ctc acc aag tcc

T K L T C L V T D L T T Y D S V
acc aag ttg acc tgc ctg gtc aca gac ctg acc acc tat gac agc gtg

T I S W T R Q N G E A V K T H T
acc atc tcc tgg acc cgc cag aat ggc gaa gct gtg aaa acc cac acc

N I S E S H P N A T F S A V G E
aac atc tcc gag agc cac ccc aat gcc act ttc agc gcc gtg ggt gag

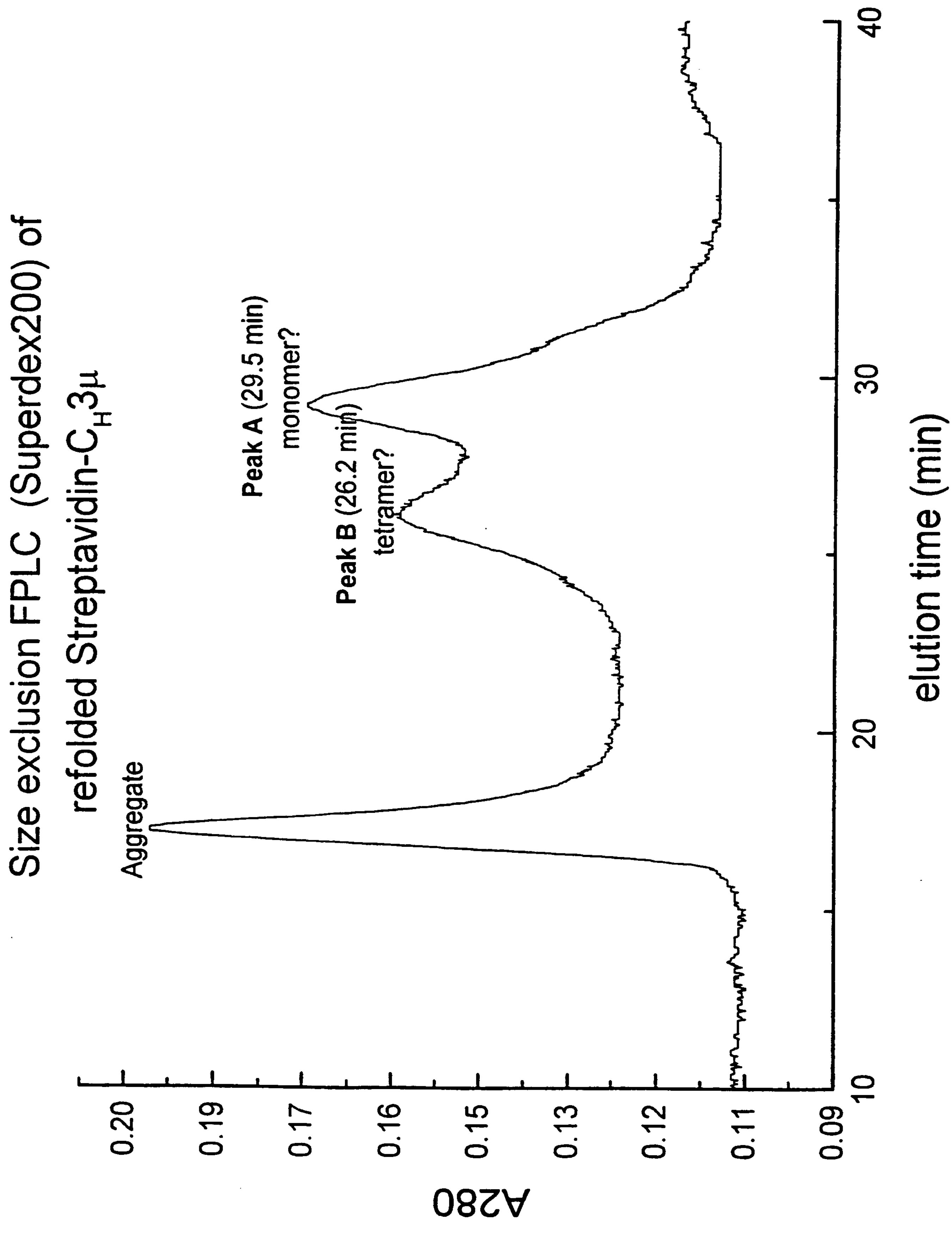
A S I C E D D W N S G E R F T C
gcc agc atc tgc gag gat gac tgg aac tcc ggg gag agg ttc acg tgc

T V T H T D L P S P L K Q T I S
acc gtg acc cac aca gac ctg ccc tcg cca ctg aag cag acc atc tcc

R P K G A A D Y K D D D D K *
cgg ccc aag ggc gcc gcg gat tat aaa gat gat gat gat aaa taa GAA
Sac 2 |------------FLAG-------------| Eco

TTC AGC CCG CCT AAT GAG CGG GCT TTT TTT TAA TTC ACT GGC CGT CGT

Figure 11


Size exclusion FPLC (Superdex200) of
refolded Streptavidin-$C_H3\mu$
Aggregate
Peak A (29.5 min)
monomer?
Peak B (26.2 min)
tetramer?
A280
0.20
0.19
0.17
0.16
0.15
0.13
0.12
0.11
0.09
10
20
30
40
elution time (min)

SEQUENCE LISTING

<110> CRC for Diagnostic Technologies

<120> Bifunctional molecules

<130> 91434

<140>
<141>

<160> 4

<170> PatentIn Ver. 2.1

SEQ ID NO: 1
<211> 203
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial sequence comprising sequence from Staphylococcal protein A fused to a sequence from human immunoglobulin

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Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln
20 25 30

Asn Ala Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln
35 40 45

Arg Asn Gly Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala
50 55 60

Asn Leu Leu Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys
65 70 75 80

Ser Asp Pro Ala Ala Ala Asp Gln Asp Thr Ala Ile Arg Val Phe Ala
85 90 95

Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser Thr Lys Leu
100 105 110

Thr Cys Leu Val Thr Asp Leu Thr Thr Tyr Asp Ser Val Thr Ile Ser
115 120 125

Trp Thr Arg Gln Asn Gly Glu Ala Val Lys Thr His Thr Asn Ile Ser
130 135 140

Glu Ser His Pro Asn Ala Thr Phe Ser Ala Val Gly Glu Ala Ser Ile
145 150 155 160

Cys Glu Asp Asp Trp Asn Ser Gly Glu Arg Phe Thr Cys Thr Val Thr
165 170 175

His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser Arg Pro Lys
180 185 190

Gly Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys
195 200

SEQ ID NO: 2
<211> 672
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial sequence comprising sequence from Staphylococcal protein A fused to a sequence from human immunoglobulin

<400> 4

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atggccgcgg ataacaaatt caacaaagaa caacaaaatg ctttctatga aatcttacat 120
ttacctaact taaacgaaga acaacgcaat ggtttcatcc aaagcctaaa agatgaccca 180
agccaaagcg ctaacctttt agcagaagct aaaaagctaa atgatgctca agcaccaaaa 240
agtgatcccg cggccgcaga tcaagacaca gccatccggg tcttcgccat ccccccatcc 300
tttgccagca tcttcctcac caagtccacc aagttgacct gcctggtcac agacctgacc 360
acctatgaca gcgtgaccat ctcctggacc cgccagaatg gcgaagctgt gaaaacccac 420
accaacatct ccgagagcca ccccaatgcc actttcagcg ccgtgggtga ggccagcatc 480
tgcgaggatg actggaactc cggggagagg ttcacgtgca ccgtgaccca cacagacctg 540
ccctcgccac tgaagcagac catctcccgg cccaagggcg ccgcggatta taaagatgat 600
gatgataaat aagaattcag cccgcctaat gagcgggctt ttttttaatt cactggccgt 660
cgttttacaa cg                                                      672
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SEQ ID NO: 3
<211> 270
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Artificial sequence comprising sequence from *S.avidini* fused to a sequence from human immunoglobulin

<400> 1

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 1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Ala Gly Ile Thr Gly Thr Trp Tyr Asn
            20                  25                  30

Gln Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp Gly Ala Leu
        35                  40                  45

Thr Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu Ser Arg Tyr Val
    50                  55                  60

Leu Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr Asp Gly Ser Gly Thr
65                  70                  75                  80

Ala Leu Gly Trp Thr Val Ala Trp Lys Asn Asn Tyr Arg Asn Ala His
                85                  90                  95

Ser Ala Thr Thr Trp Ser Gly Gln Tyr Val Gly Gly Ala Glu Ala Arg
            100                 105                 110

Ile Asn Thr Gln Trp Leu Leu Thr Ser Gly Thr Thr Glu Ala Asn Ala
        115                 120                 125

Trp Lys Ser Thr Leu Val Gly His Asp Thr Phe Thr Lys Val Lys Pro
    130                 135                 140

Ser Ala Ala Ser Asp Pro Ala Ala Ala Asp Gln Asp Thr Ala Ile Arg
145                 150                 155                 160

Val Phe Ala Ile Pro Pro Ser Phe Ala Ser Ile Phe Leu Thr Lys Ser
                165                 170                 175
```

Thr Lys Leu Thr Cys Leu Val Thr Asp Leu Thr Thr Tyr Asp Ser Val
180 185 190

Thr Ile Ser Trp Thr Arg Gln Asn Gly Glu Ala Val Lys Thr His Thr
195 200 205

Asn Ile Ser Glu Ser His Pro Asn Ala Thr Phe Ser Ala Val Gly Glu
210 215 220

Ala Ser Ile Cys Glu Asp Asp Trp Asn Ser Gly Glu Arg Phe Thr Cys
225 230 235 240

Thr Val Thr His Thr Asp Leu Pro Ser Pro Leu Lys Gln Thr Ile Ser
245 250 255

Arg Pro Lys Gly Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys
260 265 270

SEQ ID NO: 4
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<213> Artificial Sequence

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accgcgggcg ccgacggcgc cctgaccgga acctacgagt cggccgtcgg caacgccgag 180
agccgctacg tcctgaccgg tcgttacgac agcgccccgg ccaccgacgg cagcggcacc 240
gccctcggtt ggacggtggc ctggaagaat aactaccgca acgcccactc cgcgaccacg 300
tggagcggcc agtacgtcgg cggcgccgag gcgaggatca acacccagtg gctgctgacc 360
tccggcacca ccgaggccaa cgcctggaag tccacgctgg tcggccacga caccttcacc 420
aaggtgaagc cgtccgccgc tagcgatccc gcggccgcag atcaagacac agccatccgg 480
gtcttcgcca tcccccccatc ctttgccagc atcttcctca ccaagtccac caagttgacc 540
tgcctggtca cagacctgac cacctatgac agcgtgacca tctcctggac ccgccagaat 600
ggcgaagctg tgaaaaccca caccaacatc tccgagagcc accccaatgc cactttcagc 660
gccgtgggtg aggccagcat ctgcgaggat gactggaact ccggggagag gttcacgtgc 720
accgtgaccc acacagacct gccctcgcca ctgaagcaga ccatctcccg gcccaagggc 780
gccgcggatt ataaagatga tgatgataaa taagaattca gcccgcctaa tgagcgggct 840
ttttttaat tcactggccg tcgt 864

INTERNATIONAL SEARCH REPORT	International application No. PCT/AU 98/01076

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: C12N 15/00 C12N 15/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CA } WPIDS} medline} chimeric, antibody, domains, bifunctional, immunoglobulin, heavy chain, constant region

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Borrebaeck, C.A.K. (ed)., <u>Antibody Engineering (2ND ed.)</u>, (1995) Oxford University Press, Inc. see pages 205-255	1-89
X	Spooner, R.A. et al., "Genetically Engineered Antibodies for Diagnostic Pathology", <u>Human Pathology</u> (June 1994) Vol. 25 No. 6 pages 606-614 see entire document	1-89

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 February 1999	-3 MAR 1999
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	Authorized officer K.F. PECK Telephone No.: (02) 6283 2263

INTERNATIONAL SEARCH REPORT

International application No. PCT/AU 98/01076

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Wright, A. et al., "Genetically engineered antibodies: Progress and Prospects", Critical Reviews in Immunology (1992) Vol 12, No's (3, 4) pages 125-168 see particularly pages 149-158	1-89
X	Pearce, L.A. et al., "Linear Gene fusions of antibody fragments with streptavidin can be linked to biotin labelled secondary molecules to form bispecific reagents", Biochemistry and Biology International (September 1997) Vol. 42, No. 6 pages 1179-1188 see entire document	1-89
X	Coloma, M.J. et al., "Design and production of novel tetravalent bispecific antibodies", Nature Biotechnology (February 1997) Vol. 15 pages 159-163 see entire document	1-89
X	Yanamura, M. et al., "A Human/Mouse chimeric monoclonal antibody against intercellular adhesion molecule-1 for tumour radio immunoimagings" Jpn. J. Cancer Res. (April 1996) Vol. 187 pages 405-411 see entire document	1-89
X	Flamez, D. et al., "Production in Escherichia coli of a functional murine and murine:: human chimeric $F(ab')_2$ fragment and mature antibody directed against human placental alkaline phosphatase", Journal of Biotechnology (1995) Vol. 42 pages 133-143 see entire document	1-89
X	Sandhu, J.S. et al., "Protein engineering of antibodies", Critical Reviews in Biotechnology, (1992) Vol. 12 (No. 5, 6) pages 437-462 see entire document	1-89
X	Calvo, B. et al., "Construction and purification of domain-deleted immunoglobulin variants of the recombinant/chimeric B72.3(γ1) monoclonal antibody", Cancer Biotherapy (1993) Vol. 8 No. 1, pages 95-109 see entire document	1-38
X	Jin, B.R. et al., "Cloning, expression and characterization of a murine-human chimeric antibody with specificity for pre-S2 surface antigen of hepatitis B virus", Molecular Immunology (1993) Vol. 130, No. 18, pages 1647-1654 see entire document	1-38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

ving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 91434

Box No I TITLE OF INVENTION

BIFUNCTIONAL MOLECULES

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

DIATECH PTY LTD
GPO Box 2434
Brisbane
Queensland 4001
AUSTRALIA

☐ This person is also inventor.

Telephone No

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ATWELL, John Leslie
7 Glenwerri Court
Vermont South
Victoria 3133
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

F B RICE & CO
605 Darling Street
BALMAIN NSW 2041
AUSTRALIA

Telephone No

(612) 9810 7133

Facsimile No.

(612) 9810 8200

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III . URTHER APPLICANTS AND/OR (FURTHER) INVE . ORS

If none of the following sub-boxes is used, this sheet is not to be included in the request

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

DEVINE, Peter Leonard
135 Cribb Road
Carindale
Queensland 4152
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

COIA, Gregory
73 Union Street
Brunswick
Victoria 3056
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

KORTT, Alexander Andrew
23 Upland Street
Strathmore
Victoria 3041
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PERRY, Gillian Wendy
20 Gibbons Street
Werribee
Victoria 3030
AUSTRALIA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: UK

State *(that is, country)* of residence: AU

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III **FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS**

If none of the following sub-boxes is used, this sheet is not to be included in the request

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BUNDESEN, Peter Gregory
15 Games Street
Fig Tree Pocket
Queensland 4069
AUSTRALIA

This person is:

- [ ] applicant only
- [x] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: AU

State *(that is, country)* of residence: AU

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [x] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATIO. ✓F STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked:*

Regional Patent

☒ **AP** **ARIPO Patent: GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA** **Eurasian Patent: AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent: AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent: BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* ....

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

- ☒ **AL** Albania ....
- ☒ **AM** Armenia ....
- ☒ **AT** Austria ....
- ☒ **AU** Australia ....
- ☒ **AZ** Azerbaijan
- ☒ **BA** Bosnia and Herzegovina..........
- ☒ **BB** Barbados
- ☒ **BG** Bulgaria ....
- ☒ **BR** Brazil ....
- ☒ **BY** Belarus ....
- ☒ **CA** Canada
- ☒ **CH and LI** Switzerland and Liechtenstein
- ☒ **CN** China ....
- ☒ **CU** Cuba..........
- ☒ **CZ** Czech Republic..........
- ☒ **DE** Germany ....
- ☒ **DK** Denmark ....
- ☒ **EE** Estonia ....
- ☒ **ES** Spain ....
- ☒ **FI** Finland ....
- ☒ **GB** United Kingdom
- ☒ **GD** Grenada
- ☒ **GE** Georgia ....
- ☒ **GH** Ghana ....
- ☒ **GM** Gambia
- ☒ **HR** Croatia
- ☒ **HU** Hungary ....
- ☒ **ID** Indonesia
- ☒ **IL** Israel ....
- ☒ **IN** India ....
- ☒ **IS** Iceland
- ☒ **JP** Japan ....
- ☒ **KE** Kenya ....
- ☒ **KG** Kyrgyzstan ....
- ☒ **KP** Democratic People's Republic of Korea ....
- ☒ **KR** Republic of Korea ....
- ☒ **KZ** Kazakstan ....
- ☒ **LC** Saint Lucia
- ☒ **LK** Sri Lanka
- ☒ **LR** Liberia
- ☒ **LS** Lesotho ....
- ☒ **LT** Lithuania
- ☒ **LU** Luxembourg
- ☒ **LV** Latvia
- ☒ **MD** Republic of Moldova ....
- ☒ **MG** Madagascar ....
- ☒ **MK** The former Yugoslav Republic of Macedonia ....
- ☒ **MN** Mongolia
- ☒ **MW** Malawi ....
- ☒ **MX** Mexico ....
- ☒ **NO** Norway
- ☒ **NZ** New Zealand ....
- ☒ **PL** Poland ....
- ☒ **PT** Portugal ....
- ☒ **RO** Romania
- ☒ **RU** Russian Federation ....
- ☒ **SD** Sudan
- ☒ **SE** Sweden
- ☒ **SG** Singapore
- ☒ **SI** Slovenia ....
- ☒ **SK** Slovakia ....
- ☒ **SL** Sierra Leone
- ☒ **TJ** Tajikistan ....
- ☒ **TM** Tukmenistan ....
- ☒ **TR** Turkey ....
- ☒ **TT** Trinidad and Tobago ....
- ☒ **UA** Ukraine ....
- ☒ **UG** Uganda ....
- ☒ **US** United States of America.......... ....
- ☒ **UZ** Uzbekistan ....
- ☒ **VN** Viet Nam ....
- ☒ **YU** Yugoslavia..........
- ☒ **ZW** Zimbabwe ....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ ....
- ☐ ....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No VI PRIORITY CL..M ☐ Further priority claims a.. indicated in the Supplemental Box

Filing date of earlier application (day/month/year	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 24 December 1997 24/12/1997	PP1110	Australia		
item (2) 11 August 1998 11/08/1998	PP5176	Australia		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1) - (2)

**Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii). See Supplemental Box*

Box No VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:
ISA /

Request to use results of earlier search: reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:
Date *(day/month/year)* Number Country *(or regional Office)*

Box No VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	5
description (excluding sequence listing part):	40
claims	10
abstract	1
drawings:	11
sequence listing part of description	3
Total number of sheets:	70

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract: One

Language of filing of the international application:

Box No IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Jenny Petering

JENNY E. PETERING
for and on behalf of F B Rice & Co

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	☐ received
4. Date of timely receipt of the required corrections under PCT Article 11(2):	☐ not received
5. International Searching Authority (if two or more are competent): ISA/	6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

8

PATENT COOPERATION TREATY

17

REC'D 28 DEC 1999
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 91434	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 98/01076**	International filing date *(day/month/year)* 24 December 1998	Priority Date *(day/month/year)* 24 December 1997

International Patent Classification (IPC) or national classification and IPC

Int. Cl.^{6} C12N 15/00, 15/13

Applicant

DIATECH PTY LTD

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, ie., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 2 July 1999	Date of completion of the report 9 December 1999
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer I J Dowd **IAN DOWD** Telephone No. (02) 6283 2273

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of .

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been-established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-89	YES
	Claims	NO
Inventive step (IS)	Claims 1-89	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-89	YES
	Claims	NO

2. Citations and explanations (Rule 70.7)

(D1) Borrebaeck, C.A.K. (ed)., Antibody Engineering (2nd ed), (1995) Oxford University Press, Inc.

(D2) Spooner, R-A. et al., "Genetically Engineered Antibodies for Diagnostic Pathology", Human Pathology (June 1994) Vol. 25 No. 6 pages 606-614

(D3) Wright, A. et al., "Genetically engineered antibodies: Progress and Prospects", Critical Reviews in Immunology (1992) Vol 12, No's (3, 4) pages 125-168

(D4) Pearce, L.A. et al., "Linear Gene fusions of antibody fragments with streptavidin can be linked to biotin labelled secondary molecules to form bispecific reagents", Biochemistry and Biology International (September 1997) Vol. 42, No. 6 pages 1179-1188

(D5) Coloma, M.J. et al., "Design and production of novel tetravalent bispecific antibodies", Nature Biotechnology (February 1997) Vol. 15 pages 159-163

(D6) Yamamura, M. et al., "A Human/Mouse chimeric monoclonal antibody against intercellular adhesion molecule- 1 for tumour radio inimunoimagings" Jpn. J. Cancer Res. (April 1996) Vol. 187 pages 405-411

(D7) Flamez, D. et al., "Production in Escherichia coli of a functional murine and murine::human chimeric $F(ab')_2$ fragment and mature antibody directed against human placental alkaline phosphatase", Journal of Biotechnology (1995) Vol. 42 pages 133-143

(D8) Sandhu, J.S. et al., "Protein engineering of antibodies", Critical Reviews in Biotechnology, (1992) Vol. 12 (No. 5, 6) pages 437-462

(D9) Calvo, B. et al., "Construction and purification of domain-deleted immunoglobulin variants of the recombinant/chimeric B72.3(yl) monoclonal antibody", Cancer Biotherapy (1993) Vol. 8 No. 1, pages 95-109

(D10) Jin, B.R- et al., "Cloning, expression and characterization of a murine-human chimeric antibody with specificity for pre-S2 surface antigen of hepatitis B virus", Molecular Immunology (1993) Vol. 130, No. 18, pages 1647-1654

The present invention relates to chimeric binding moieties which are useful as positive control reagents in diagnostic tests for detecting infectious diseases. As explained in the "Background of the Invention" section of the specification, diagnostic tests for the detection of infectious microorganisms in humans require positive control reagents which enable the user to verify that the test conditions are correct. These positive control reagents must possess the following features: 1.A binding region, such as an antibody Fab fragment, which binds to an antigen derived from the infectious microorganism; and 2.an epitopic region which is recognised by class specific anti-human immunoglobulin antisera (e.g. a human antibody Fc fragment).

Continued

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : Box V

In the prior art, such positive control reagents have been produced by grafting Fab fragments from mouse monoclonal antibodies (which have been raised against antigens derived from the infectious microorganism) onto human Fc fragments. The production of these chimeric antibody reagents is cumbersome in that it involves manipulation of relatively large Fc fragments.
The present invention is based on the finding that only small regions of the human Fc fragment (e.g. individual C_H domains) are sufficient to provide epitopic fragments recognised by class specific anti-human antisera. That is, these individual C_H domains work effectively on recognition sites for anti-human immunoglobulin antisera.
Claims 1 to 38 relate to the chimeric constructs of the present invention comprising antigenic binding regions (eg Scfv fragments) linked to individual C_H domains, thus avoiding the inconvenience of manipulating full length or "naturally occurring" Fc fragments. Moreover, the chimeric constructs of the present invention may be readily produced by recombinant DNA technology. This aspect of the invention is illustrated in Figure 2.
Claims 39 to 89 relates to a chimeric construct which can be used to decorate or label mouse antibodies, for example, with human C_H domains. This chimeric construct comprises a C_H domain linked to a molecule (such as *Staphylococcus aureus* protein A) which is able to bind to an antibody molecule (as depicted in Figure 3). Alternatively, the construct comprises a C_H domain linked to a molecule (such as streptavidin) which is able to bind to a group provided on the antibody (such as biotin) (as depicted in Figure 4). Once the chimeric construct is bound to the antibody it gives rise to a complex which has the properties of a specific positive antibody control: a ligand binding site with specificity for an antigen derived from the infectious microorganism (eg. the Fab fragment of the antibody); and an epitopic domain which is recognised by antisera (eg. the C_H domain portion of the chimeric molecule bound to the antibody).
The present invention provides two approaches for generating positive control reagents which are useful in diagnostic tests. These two approaches are based on the finding that only minimal regions from antibody constant (Fc) fragments are sufficient for recognition by class specific antisera. The present inventors have also found that the C_H3 domain provides enhanced reactivity in ELISA assays than other C_H domains.

With reference to the citations listed previously, the Attorney has elucidated the differences between the present application and the citations.

(D1) relates to the production and expression in *E. coli* of scFv or Fab antibody fragments, some with heterologous C-terminal tails related to purification, or expression and bacteriophage display. There is no disclosure of chimeric constructions comprising scFv fragments from one antibody linked to a constant domain from another antibody (as claimed in present claims 1 to 38) and nothing about the use of any constructs as positive control reagents in diagnostic tests. On page 213 a bifunctional construct comprising Fragment B of protein A joined to an scFv is described, These constructs do not fall within the scope of the present claims. The bifunctional linker constructs defined in claims 39 to 89 encompass Fragment B of protein A joined to an inununoglobulin constant domain. Such a construct is not described in either of these chapters, and there is nothing to teach the possibility of using such a bifunctional molecule in diagnostic tests as claimed in the present application.

(D2) describes genetically engineered antibodies and fragments for *in vivo* diagnosis and therapy, diagnostic histopathology and immunohistochemistry. In figure 1 there is a depiction of a chimeric molecule, in which an scFv is joined to another effector function. The figure legend describes novel effector functions, such as enzymic activities may be fused to a scFv at the free carboxy terminus However, neither the review or the references cited describe a construct comprising an scFv fragment from one antibody linked to a constant domain from another antibody(as defined in present claims 1 to 38) or its use as a diagnostic control.
The citation also describes the expression of recombinant streptavidin in *E. coli* in the form of a biotin binding tetramer, a biotin tetrameric protein A fusion protein, and in the form of an scFv-streptavidin fusion (see page 610). There is no description, however, of construct comprising streptavidin joined to an immunoglobulin constant domain as required by claims 39 to 89 of the present specification.

(D3) is a review of progress made in genetically engineered antibodies. The paper describes humanisation of antibodies (e.g. where CDR regions from murine antibodies are grafted onto human scFv domains) and chimeric antibodies comprising murine scFv fragments linked to human Fc fragments. This review does not address the problem faced by the present inventors which is to produce an improved positive control antibody reagent for use in diagnostic tests. Rather, this paper addresses the problems involved with using murine antibodies as therapeutic agents.

Continued

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box V

These problems include short half-life; immunogenicity and lack of effective interaction with the host immune system. This citation does not disclose chimeric constructs comprising scFv molecules linked to immunoglobulin constant domains, or constructs which enable antibodies to be labelled with C_H domain as claimed in the present application.

(D4) discloses a chimeric construct comprising an scFv linked to a streptavidin domain. This construct does not fall within the scope of claims 1 to 38 which define chimeric constructs comprising scFv- C_H fusions. Nor do they fall within the scope of claims 39 to 89 which define Streptavidin- C_H domain chimeric constructs. This citation is directed to a different problem to that of the present application. The purpose of the streptavidin in the scFv chimera disclosed in (D4) is to promote multimerisation of the scFv so as to increase avidity of the reagent. It does not teach nor suggest the use of streptavidin as a joining domain to decorate or label whole mouse antibody with human C_H domains.

(D5) is directed to a completely different problem to that of the present application. The citation does not relate to the use of positive antibody control reagents in diagnostic tests. Rather, it is directed to the production of bispecific antibody molecules which simultaneously bind to two different antigens such as a tumour cell antigen and a drug or effector cell. This citation does not relate to the production of a chimeric positive antibody control reagent comprising an antibody binding region derived from a non-human (eg. a murine scFv fragment) linked to a constant domain (eg. a C_H3 domain from a human antibody).

(D6) describes an expression vector encoding only a mouse V_H region and a human constant region. It does not encode a V_L region nor does it encode a flexible linker region to act as a hinge between the V_H and V_L regions. In the case of the Yamamura citation, the antibody is produced by transfecting mutant cells which produce only murine V_L chains with the expression vector encoding the V_H and constant region chains. Accordingly, this citation does not disclose a polynucleotide molecule comprising all of the essential features of present claim 13 or claim 20. (D6) does not disclose a diagnostic method (as claimed in claims 33 to 38) which involves the use of a conjugate of claim 1 as a positive control. With regard to claims 39 to 50(D6) does not disclose a construct which has a domain which binds to a non- human antibody for the purpose of decorating that antibody with a human CH domain.

(D7) relates to the expression in E. coli of intact murine:human chimeric antibody IgG_3 and corresponding Fab'$_2$ fragments. The aim of these experiments is to reduce immunogenic reactions when compared to intact mouse mabs after injection into humans for cancer therapy. There is nothing in this citation to suggest the production of chimeric antibody constructs for use as positive control reagents in diagnostic tests for infectious diseases.

(D8) is a review of antibody engineering. Chimeric antibodies are discussed in general at page 443 of the publication. Notably, these chimeric antibodies comprise full length Fc fragments (see Figure 3). These antibodies do not fall within the scope of the present claims. Antibody fusion proteins are described at page 454 of the citation. These fusion proteins are produced by either eliminating the Fc fragment and replacing it with a molecule which has a new biological function (i.e. polymerase or nuclease activity); or by fusing the Fc fragment with a molecule which has a new biological function (e.g. CD4 fused to an antibody constant domain). This publication does not disclose the scFV-C_H chimera constructs defined in claims 1 to 38. Nor does it disclose a chimeric molecule comprising a C_H domain and a region which binds to an antibody as claimed in claims 39 to 89.

(D9) describes constant domain-deleted chimeric antibodies produced in mammalian cell culture. Domains were deleted in order to improve tissue penetration when targeting tumours in vivo and to reduce HAMA response when injected into patients. This citation does not address the problem of producing positive antibody control reagents for detecting infections microorganisms.

(D10) relates to the construction of a mouse/human chimeric antibody for the purpose of reducing HAMA response following injection of the antibody into humans. This citation does not address the issue of producing positive antibody control reagent.